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SHORT COMMUNICATIONS

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Postembryonic mushroom body development in a migrating butterfly, *Vanessa cardui* (Lepidoptera: Nymphalidae)

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ABSTRACT: Behavioural changes in adult insects may correspond to neural plasticity in their brain. The aim of the present work is to study if neurogenesis exists during adult life in the brain of the butterfly *Vanessa cardui* which exhibits large scale migration in spring and autumn. Larvae, pupae and adults were injected with a S-phase marker, 5-bromo-2′-deoxyuridine (BrdU) and animals were sacrificed at different periods thereafter. No neurogenesis could be found during adulthood of *V. cardui*. Mushroom body neuroblasts are dissappearing during the pupal stage. During the larval stages numerous Kenyon cells, originating from neuroblast proliferation appear in the mushroom bodies. Most of them disappear during metamorphosis. Giant glial cells proliferate mainly during the first half of the pupal stage.

KEYWORDS: Adult neurogenesis, neuroblasts, mushroom bodies, glial cells, migratory behaviour, *Vanessa cardui*

INTRODUCTION

Mushroom bodies are the main integrative structures of insect brain and probably play a major role in the adaptation of behavioural responses to the environment. They receive sensory informations from the eyes, the palps, and the antennae and play an important role in processing and storage of the sensory informations (for review see Hammer and Menzel, 1995; Heisenberg, 1998). From the discovery of Dujardin (1850), the mushroom body structure has been widely investigated (for review see Strausfeld *et al.*, 1998). Briefly, this paired structure located in the protocerebrum, consists of densely packed intrinsic neurons, the Kenyon cells and differentiated neuropils. The neuropilar parts comprise a cup-like structure, the calyx. A correlation

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between the structure of mushroom bodies and the complexity of behaviour has been hypothesized for many years (Rensch, 1959; Howse, 1975), but mushroom body functions are not clearly understood and their roles in behaviour remain largely unanswered.

Although insects are considered to have a rather inflexible central nervous system, mushroom body development is not achieved at the end of embryogenesis but continues throughout the preimaginal stages. During preimaginal development, neuroblasts can be observed in the corpora pedunculata of all insect species, but differences occur in their distribution. In a study of insect brain postembryonic development, Panov (1957) distinguished orders including Neuroptera, Diptera, and Lepidoptera, which contain isolated or scattered neuroblasts (1 to 20 per calyx), whereas aggregated neuroblasts characterize other orders such as Ensifera, Caelifera, Hymenoptera, and Coleoptera. However, species specific differences can be observed. Neuroblasts are dividing either symetrically to produce new neuroblasts, or asymetrically to give birth to smaller ganglion mother cells, which further divide once giving rise to two Kenyon cells (Panov, 1957, 1962; Nordlander and Edwards, 1970; Pipa, 1978).

In some insect species, neuroblasts still proliferate during the imaginal life; such is the case for various Gryllidae as the house cricket, *Acheta domesticus*, some Coleoptera, for *Oncopeltus fasciatus* (Cayre *et al.*, 1994, 1996), and for *Mantis religiosa* (unpublished observations Cayre *et al.*). This secondary neurogenesis seems to be modulated by internal (e.g. juvenile hormones stimulated mushroom body mitotic activity in *A. domesticus* whereas allatectomy reduced the number of cell divisions; Cayre *et al.* 1994) as well as external factors (Strambi *et al.*, 1999; Lomassese *et al.*, 2000). For instance in *A. domesticus,* environmentally enriched animals were found to have an increased number of new born neurons in their mushroom bodies compared with crickets housed in cages with an impoverished environment. In the honeybee (*Apis mellifera*) flight, nursing and foraging experiences modulate dendritric spine morphology in the mushroom body calyx (Brandon and Coss, 1982; Farris *et al.*, 2001). Furthermore, it has been shown that foraging bees present increased mushroom body neuropile volume compared with nursing bees (Withers *et al.*, 1993). This increase in size of mushroom body, however, seems not to be a consequence of neurogenesis (Fahrbach *et al.*, 1995).

The aim of the present work was to examine if neurogenesis exists during adult life in the mushroom bodies of the butterfly *Vanessa cardui*. This species shows drastic changes in behaviour after metamorphosis. Adults exhibit large scale migration in spring from Southern Europe or Northern Africa to Middle and Northern Europe to establish one to several generations there. Overwintering in Middle Europe has never been observed and it is assumed that in late summer, butterflies return South for overwintering in a reproductive diapause (Ebert and Rennwald, 1993).

MATERIALS AND METHODS

Animals

Adults species of *Vanessa cardui* were caught in the field in springtime and brought into the laboratory for oviposition. Hatched larvae were reared on a semi-artificial diet (slightly modified after Nijhout, 1980 by adding leaf powder of *Cirsium arvense* instead of *Plantago* sp.) at 25 °C under long-day conditions (16L : 8D). Imagines were allowed to feed from a 10% sucrose solution.

BrdU labelling

DNA replication was monitored using 5-bromo-2′-deoxyuridine (BrdU, Sigma), a thymidine analog incorporated during DNA synthesis, and detected immunohistochemically by using a monoclonal antibody against BrdU.

For *in vivo* incorporation, either larvae, pupae or newly emerged adults received one single 4 μl abdominal injection of a BrdU solution (40 mg/mL in saline) and, were then maintained in their respective environment up to dissection. Most of the insects were dissected as adults, but in some cases, injected larvae were examined 2 days after BrdU injection.

To evaluate BrdU incorporation, cerebral ganglia were rapidly dissected under saline and fixed for 2 hrs in Carnoy's fixative (absolute ethanol : chloroform : acetic acid/6 : 3 : 1/v,v,v). The tissues were then rehydrated in PBS-TX (0.1 M phosphate buffer, pH 7.4, 0.15 M NaCl, 0.3% Triton X-100). DNA hydrolysis proceeded for 1 hr in 2 N HCl in PBS-TX at room temperature, followed by three 15 min rinses in PBS-TX (Lomassese *et al.*, 2000). Depending upon the quality of the primary antibody, it was sometimes necessary to treat the samples with trypsin (0.01% in saline for 25 min at 37 °C). Cerebral ganglia were then rinsed in distilled water and passed through graded alcohols. Finally, they were embedded in paraffin and 6 μm serial sections were performed.

Tissue sections were deparaffinized in toluene, passed through absolute alcohol, and rehydrated in distilled water. After rinsing in PBX-TX, they were incubated at 4 °C for 1 hr in 5% normal goat serum in PBS-TX-BSA (PBS-TX containing 0.2% bovine serum albumin), before being exposed overnight at 4 °C to a 1/100 dilution of anti-BrdU antiserum (Dako, Trappes, France) in PBS-TX-BSA. After careful washing in PBS-TX, incubation took place in goat anti-mouse IgG antibody conjugated with peroxidase (1/3000 in PBS-TX; Biorad, Hercules, CA) for 3 hrs at 4 °C. Peroxidase detection was performed using 3,3'-diaminobenzidine (Sigma).

RESULTS

Labelling and location of cells generated during the larval instars

In larvae injected during the first half of the 4th larval instar and dissected two days later, mushroom labelled nuclei could be visualized in the brain (Fig. 1). Interestingly, in mushroom bodies, large cells at the superior part of the cortex were present. According to the mushroom body sample, their number varied between 20 and 45.

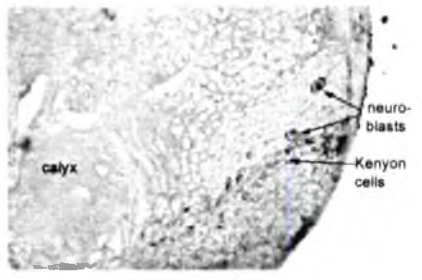

Fig. 1

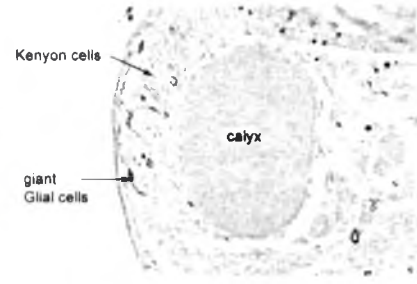

Fig. 2

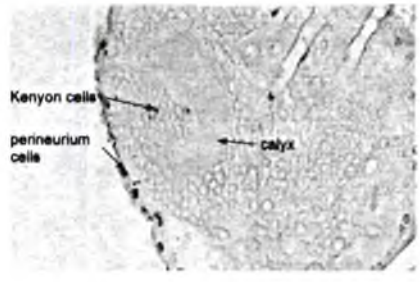

Fig. 3

FIGURE 1. 5-Bromo-2′-deoxyuridine (BrdU)-labelled neuroblasts in the brain of *V. cardui* injected in the early 4th larval instar and dissected 2 days later. Magnification 40-fold.

FIGURE 2. 5-Bromo-2′-deoxyuridine (BrdU) labelling of *V. cardui* brain at the end of the 4th larval stage and dissected as young adult. Magnification 40-fold.

FIGURE 3. 5- Bromo-2′-deoxyuridine (BrdU) labelling of *V. cardui* brain in the middle of the pupal stage and dissection as young adult. Magnification 40-fold.

Most of them were still containing BrdU and presented a strong immunoreactivity. These large cells can be considered as neuroblasts.

The Kenyon cells appeared as small cells densely packed and arranged in narrow parallel rows. Two days after BrdU injection, a central group of 500 to 600 labelled Kenyon cells could be observed occupying the median area of the cortex. These cells were lying in parallel columns between the superior part of the cortex and the calyx neuropile and represent neuroblast progeny. Their large number indicated that neuroblasts were actively dividing during the two days following BrdU injection.

Whereas more than 500 labelled Kenyon cells were present 2 days after BrdU injection into young 4th instar larvae, only few of them could be observed scattered in mushroom body cortex when dissection occurred at the adult stage. For larvae injected at the beginning of the 4th instar, about 60 labelled Kenyon cells were present in each mushroom body cortex, and only 50 when labelling occurred later in the instar, indicating that mushroom body neuroblast proliferation decreased during the first half

of the instar. Some few labelled Kenyon cells could be observed in the calyx of larvae that had been injected at the end of the 4th instar and dissected as adults, but most of the label occurred in glial cells and especially in giant glial cells surrounding mushroom body neuropiles or scattered in the brain (Fig. 2).

When larvae were injected at the end of the 5th instar and dissected as adults, the results were similar to those described for the 4th larval instar. Only few surviving labelled Kenyon cells could be observed. Some giant glial cells and smaller glial cells surrounding mushroom body neuropiles were immunoreactive.

Injection into prepupae and pupae

When BrdU had been injected into prepupae and pupae and animals were dissected as adults, most of the label concerned glial cells and especially giant glial cells. A strong immunoreactivity was observed in the perineurial nuclei when labelling occurred in the middle of pupal stage (Figure 3). Injections at the end of pupal stage resulted only in the label of some few perineurium nuclei.

Injection into adults

BrdU injection into newly emerged adult females failed to result in any label in the brain structures, indicating that in this species neurogenesis only occurs during preimaginal development.

DISCUSSION

The data in the present work allow to clarify some characteristics of the brain postembryonic development in *V. cardui*: (i) There is no neurogenesis during the adulthood of *V. cardui*, although the imagines exhibit large scale migrations which may demand special behaviour abilities and adaptation of behavioural responses to the environment; (ii) Mushroom body neuroblasts particularly proliferate during the early 4th larval instar, but are disappearing during the pupal stage; (iii) During the larval stages numerous Kenyon cells, originating from neuroblast proliferation appear in the mushroom bodies. Most of them disappear during metamorphosis; (iv) Giant glial cells seem to proliferate during the first half of pupal stage. These results more or less agree with findings previously described in other Lepidoptera and especially in another migrating species, the monarch butterfly *Danaus plexippus* (Nordlander and Edwards, 1969).

Usually, the spindle axis of dividing neuroblasts is perpendicular to the neuropile and the new born cells are lying between the neuroblasts and the neuropile. Thus, the final arrangement after several divisions of the neuroblasts is a row of new born Kenyon cells. Such is the case for *V. cardui* when dissection occurred some few hours after BrdU injection into 4th instar larvae. As described in other insect species (Nordlander and Edwards, 1970; Farris *et al.*, 1999; Cayre *et al.*, 2000), when brains are fixed after successively larger intervals, the labelled cells appear increasingly farther from the neuroblasts, forming 'growth rings' according to the terminology of

Weiss and Edwards (1974). As in other Lepidoptera described so far, all neuroblasts in the brain of *V. cardui* have degenerated through the pupal period, as most of the label observed in samples injected as pupae and dissected as adults corresponded to glial cells. By contrast to the conclusions of Nordlander and Edwards (1969) who estimated that the majority of larval brain cells were incorporated into the adult brain of *D. plexippus*, our data indicate massive disappearance of neurons born during the larval life. When up to 600 labelled Kenyon cells could be numbered two days after BrdU injection into young 4th instar larvae, about 10% only could be observed in the resulting adults. Large morphological changes are occurring in the lepidopteran brain during metamorphosis: whereas mushroom bodies present a single calyx in larvae, they possess a double calyx in adults. Moreover, the well developed mushroom body cortex of larvae is drastically reduced in the adult indicating massive disappearance of larval neurons.

In their description of postembryonic brain development of *D. plexippus*, Nordlander and Edwards (1969) distinguished several types of glial cells, among them glial I type corresponding to perineurial cells, glial III type corresponding to giant glial cells, and glial IV type to neuropile sheath cells. The temporal distribution of proliferation of glial cells can be distinguished from that of mushroom body neuroblasts. As described in our results, if some few giant glial cells are incorporating BrdU in larvae injected at the end of the 4th instar, their period of intense proliferation corresponded to the end of the 5th larval instar and the first half of the pupal stage. These data are in agreement with those observed in *D. plexippus* (Nordlander and Edwards, 1969). Small type IV glial cells seem to proliferate, as in *D. plexippus*, during a short period on either side of ecdysis. The main proliferative period for glial I type cells seems to correspond to the end of the pupal stage. Concerning the origin of glial cells, this preliminary study did not allow to conclude. It can be hypothesized that stem cells present during embryogenesis or during the first larval instars could give birth to neuroblasts and glia. However, it must be underlined that glial III type cells differ from other glial types by mainly proliferating at metamorphosis, and these giant cells seem to become polyploid during larval stages.

Although adult neurogenesis is not involved in the behavioural changes leading to migration of *V. cardui*, it must be remembered that, among the insect species devoid of adult neurogenesis, the outgrowth of the intrinsic neurons in mushroom bodies results in a large plasticity of the adult nervous system. Such is the case in *Drosophila melanogaster* where the fibre number in the mushroom body peduncle depends on age, sex and experience (Technau, 1984). Similarly, in the honeybee, the cytoarchitectural complexity of neurons in the mushroom bodies of adults increases as a function of age. Moreover, foraging promotes additional dendritic branching and growth (Farris *et al.*, 2001). Despite the numerous studies devoted to mushroom bodies during the last past years, we are far to understand the functional significance and the involvement of mushroom bodies in adult specific behaviour. The present data represent a first step which could help to compare mushroom body development from a phylogenetic point of view.

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REFERENCES

Brandon, J. G. and Coss, R. G. (1982) Rapid dendritic spine stems shortening one-trial learning: the honeybee's first orientation flight, *Brain Res.* **252**: 51–61.

Cayre, M., Strambi, C. and Strambi, A. (1994) Neurogenesis in an adult insect brain and its hormonal control, *Nature* **368**: 57–59.

Cayre, M., Strambi, C., Charpin, P., Augier, R., Meyer, M. R., Edwards, J. S. and Strambi, A. (1996) Neurogenesis in adult insect mushroom bodies, *J. Comp. Neurol.* **371**: 300–310.

Cayre, M., Malaterre, J., Charpin, P., Strambi, C. and Strambi, A. (2000) Fate of neuroblast progeny during postembryonic development of mushroom bodies in the house cricket, *Acheta domesticus*. *J. Insect Physiol.* **46**: 313–319.

Dujardin, F. (1850) Mémoire sur le système nerveux des insectes, *Ann. Sci. Naturell. Zool.* **14**: 195–206.

Ebert, G and Rennwald, E. (1993) *Die Schmetterlinge Baden-Württembergs. Band 1: Tagfalter*, Ulmer: Stuttgart.

Fahrbach, S. E., Strande, J. L. and Roninson, G. E. (1995) Neurogenesis is absent in the brain of adult honeybees and does not explain behavioural neuroplasticity, *Neurosci. Lett.* **197**: 145–148.

Farris, S. M., Robinson, G. E., Davis, R. L. and Fahrbach, S. E. (1999) Larval and pupal development of the mushroom bodies in the honey bee *Apis mellifera*, *J. Comp. Neurol.* **414**: 97–113.

Farris, S. M., Robinson, G. E. and Fahrbach, S. E. (2001) Experience- and age-related outgrowth of intrinsic neurons in the mushroom bodies of the adult worker honey bee, *J. Neurosci.* **21**: 6395–6404.

Hammer, M and Menzel, R. (1995) Learning and memory in honeybees, *J. Neurosci.* **15**: 1517–1630.

Heisenberg, M. (1998) What do the mushroom bodies do for the insect brain? An introduction, *Learning and Memory* **5**: 1–10.

Howse, P. E. (1975) Brain structure and behavior in insects, *Annu. Rev. Entomol.* **20**: 359–379.

Lomassese, S. S., Strambi, C., Strambi, A., Charpin, P., Augier, R., Arouane, A. and Cayre, M. (2000) Influence of environmental stimulation on neurogenesis in the adult insect brain, *J. Neurobiol.* **44**: 162–171.

Nijhout, H. F. (1980) Pattern formation on lepidopteran wings: determination of an eyespot, *Dev. Biol.* **80**: 267–274.

Nordlander, R. H. and Edwards, J. S. (1969) Postembryonic brain development in the monarch butterfly *Danaus plexippus plexippus* L. I. Cellular events during brain morphogenesis, *Wilhelm Roux's Arch* **162**: 197–217.

Nordlander, R. H. and Edwards, J. S. (1970) Postembryonic brain development in the monarch butterfly, *Danaus plexippus plexippus* L. III. Morphogenesis of centers other than the optic lobes, *Wilhelm Roux's Arch.* **164**: 247–260.

Panov, A. A. (1957) The structure of the brain at successive stages of postembryonic development, *Entomol. Rev. S.S.S.R.* **36**: 269–284.

Panov, A. A. (1962) The nature of cell reproduction in the central nervous system of the nymph of the house cricket (*Gryllus domesticus* L., Orth., Insecta), *Dokl. Akad. Nauk S.S.S.R.* **143**: 471–474.

Pipa, R. L. (1978) Patterns of neural reorganization during postembryonic development of insects, *Int. Rev. Cytol.* (Suppl. 7): 403–438.

Rensch, B. (1959) Trends towards progress of brain and sex organs, *Cold Spring Harbor Symp. Quant. Biol.* **24**: 291–303.
Strambi, C., Cayre, M. and Strambi, A. (1999) Neural plasticity in the adult insect brain and its hormonal control, *Int. Rev. Cytol.* **190**: 137–174.
Strausfeld, N. J., Hansen, L., Ki, Y., Gomez, R. and Ito, K. (1998) Evolution, discovery, and interpretations of arthropod mushroom bodies, *Learning and Memory* **5**: 11–37.
Technau, G. M. (1984) Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience, *J. Neurogenet.* **1**: 113–126.
Weiss, M. J. and Edwards, J. S. (1974) 'Growth rings' in the corpora pedunculata of the insect brain: an autoradiographic and silver impregnation analysis, *Amer. Zool.* **14**: 1253.
Withers, G. S., Fahrbach, S. E. and Robinson, G. E. (1993) Selective neuroanatomical plasticity and division of labour in the honeybee, *Nature* **364**: 238–240.

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Evidence for adipokinetic activity in the plant bug *Iphita limbata* Stal. (Pyrrhocoridae: Heteroptera)

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ABSTRACT: Investigations were carried out to detect the presence of adipokinetic factors in the neuronal tissues (brain-corpora cardiaca-corpora allata complex) of the non-flying plant bug, *Iphita limbata*. Adipokinetic response of the fat body to native hormones and to synthetic locust adipokinetic hormone-I (Lom AKH-I) was measured in both *in vivo* and *in vitro* conditions. The hyperlipaemic activities of the hormones were found to be dose-dependent. A concentration of 1.0 gland pair equivalent of the CC-extract gave maximum lipid release both *in vitro* and *in vivo* (71% and 91% stimulation respectively). With syn AKH-I, maximum hyperlipaemia was effected with 100 pmoles both *in vitro* (47% stimulation) and *in vivo* (61% stimulation). Electrophoretic studies conducted on the changes in the pattern of proteins and lipoproteins of the haemolymph accompanying hyperlipaemia, revealed that the hormones affect the transformation of some of the existing proteins and/or lipoproteins. In the high MW area, new bands appeared with a concomitant disappearance of some of the low MW bands. The results thus indicate the presence of hyperlipaemic activity and the associated interconversions of haemolymph lipoproteins in non-flying insects also, and that this is possibly associated with activities other than flight.

KEYWORDS: Corpora cardiaca, corpora allata, adipokinetic hormone (AKH), Lom AKH-I, haemolymph lipoproteins, hyperlipaemia

INTRODUCTION

Energy requirement for flight and other such sustained activities in insects is met by lipids or carbohydrates released from the fat body. This release is mediated by neuropeptide hormones belonging to the adipokinetic hormone—red pigment concentrating hormone (AKH-RPCH) family, released from the corpora cardiaca (CC), which is the most important neurohaemal organ in insects. Insect CC contains either or both adipokinetic and hyperglycaemic hormones (AKH and HGH) along with many other bioactive peptides. The presence of adipokinetic factors in the

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corpora cardiaca were first detected in the locusts, *Schistocerca gregaria* (Mayer and Candy, 1969) and *Locusta migratoria* (Beenakkers, 1969). More than 35 such peptide hormones have been isolated from different insect species (see Gaede, 1991,1992 for reviews). In many cases both adipokinetic and hyperglycaemic activities are associated with a single hormone as has been shown in *Helicoverpa zea* (Jaffe *et al.*, 1988) and in *Tabanus* sp. (Woodring and Leprince, 1992). It was believed earlier that AKHs are adult hormones, involved in the release of lipids during sustained flight of insects such as locusts, where sugars and amino acids present in the haemolymph can not meet the whole requirement (Ziegler, 1979). However, AKHs have been detected in non-flying species such as *Romalea microptera* (Spring and Gaede, 1987) and nymphal stages of the locust, *S. gregaria* (Gokuldas *et al.*, 1988). Adipokinetic hormones are now shown to be multifunctional. Besides their adipokinetic activity, they inhibit synthesis of proteins (Carlisle and Loughton, 1979, 1986; Asher *et al.*, 1984), fatty acids (Gokuldas *et al.*, 1988) and mRNA (Kodrik and Goldsworthy, 1994) and stimulate fatty acid oxidation by flight muscle (Robinson and Goldsworthy, 1977; Wheeler *et al.*, 1986). Adipokinetic activity was also found to be accompanied by a characteristic transformation of lipoproteins in the haemolymph. In the locusts, lipid mobilisation induced either by flight or by the injection of AKH-I into resting insects, resulted in a significant shift in the haemolymph protein pattern involving lipoprotein-protein interactions (Beenakkers *et al.*, 1981, 1985; Van der Horst, 1983; Goldsworthy and Wheeler, 1984). A new diacylglycerol carrying lipoprotein, A^+ is formed in the haemolymph during increased lipid mobilisation by the combination of an already existing lipoprotein, A_{yellow} with a non lipid-carrying C_L protein (Wheeler, 1981; Wheeler and Goldsworthy, 1983). The compositional changes of these various proteins during the transformation have been worked out with details of their molecular weight in various insects (Van Antwerpan *et al.*, 1988; Surholt *et al.*, 1988; Shapiro *et al.*, 1988).

In the present paper, evidence for the presence of adipokinetic factors in the neuronal tissues of the non-flying plant bug, *Iphita limbata* and the activity of these factors in mobilising lipids from the fat body and affecting interconversion of haemolymph proteins have been obtained from studies involving bioassays and polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

I. limbata were collected locally and maintained in the laboratory in glass jars on germinating green gram. Insects for experiments were maintained in the laboratory conditions at least for two days before being used. Synthetic locust adipokinetic hormone-I (Lom AKH-I, Peninsula Laboratories, USA): a stock solution of the hormone was prepared with a final concentration of 200 pmoles/μl. Various working concentrations were prepared from this stock solution; HEPES buffer (NaCl, 10 mM; KCl, 12 mM; Na_2HPO_4, 1 mM; $CaCl_2$, 1 mM; HEPES, 30 mM; sucrose, 5 mM; BSA, 2%, pH 7.2); phosphovanillin reagent (concentrated *o*-phosphoric acid and 0.525% aqueous vanillin mixed in the ratio 3 : 2 v/v); chloroform; methanol; sodium

chloride (1.0 M); acrylamide solution (acrylamide 30 g and bisacrylamide 0.8 g in 100 ml distilled water); TEMED; Tris buffer I (0.614 M, pH 8.8, containing 0.164% SDS); Tris buffer II (0.147 M, pH 6.8, containing 0.108% SDS); chamber buffer (pH 8.3, containing Tris, 0.025 M; glycine, 0.192 M; and SDS, 0.1%); sample buffer (a mixture of SDS, 0.75 g; glycerol, 1.87 ml; EDTA, 0.3 ml; 0.25 M tris-HCl, pH 6.8, 0.5 M and Bromophenol blue, 0.5%); ammonium persulphate solution (1.5%); Coomassie brilliant blue stain (G-250) (0.06% [w/v] in methanol : acetic acid: water [44 : 12 : 44] for staining gel and 0.05% [w/v] in a mixture of aqueous ethanol, 24% and *o*-phosphoric acid, 42.5% for protein quantitation); gel destaining solution (Methanol : acetic acid : water 2.5 : 37.5: 437.5 v/v); standard protein molecular weight markers (MW 205, 97.4, 68, 43 and 29 kD).

Preparation of hormone extract

Whole brain with corpora cardiaca/corpora allata complex were removed from many adult insects under a microscope and the hormone was extracted using distilled water (5 μl/gland) in a steam bath. Tissue debris was removed by spinning the extract at 8000 × g in a microfuge. Different concentrations of this extract (referred to as CC-extract) were prepared using double glass-distilled water. Concentration is expressed as gland pair equivalent (gpe).

***In vitro* incubations**

For *in vitro* experiments, fat body was removed from individual insects, washed in saline, blotted and chopped into fine pieces using a sharp razor blade on a polyvinyl disc. The minced fat body was mixed and divided into two halves, weighed and put into incubation vials containing the incubation buffer (200 μl) and different concentration of the hormone (extract, 0.001, 0.01, 0.1, 1.0 and 2.0 gpe or syn AKH-I with concentrations of 0.05, 0.075, 0.10, 0.125, 0.25, 0.5 and 1.0 μM, all in 10 μl volume) in experimentals or same volume of distilled water in controls. Fat body was mixed with the incubation mixture and incubations were carried out for 30 min in a shaker water bath maintained at 37 °C.

***In vivo* experiments**

In the case of *in vivo* experiments, same quantity of the hormones as in the *in vitro* experiments were injected into the haemocoel of the insect through the pleural membrane on the lateral side of the abdomen using a Hamilton microlitre syringe. Assuming that the average total haemolymph volume of the insect is approximately 200 μl, the final concentrations of the syn hormone and extract injected (in 5 μl volume) were considered to be same as that in the *in vitro* incubations. Haemolymph samples (5 μl) from different insects were collected using drawn-out and calibrated Pasteur pipettes before and after 30 min of injection of the hormone. Similarly, haemolymph samples were collected from distilled water injected insects which served as controls. For the purpose of collecting samples for electrophoresis,

100 pmole/10 μl syn. AKH I were used for injection and 10 μl haemolymph samples were collected.

Quantitation of lipids

After *in vitro* incubation, lipids released into the incubation medium were quantitated. Samples of the incubation medium were drawn and lipids were extracted by a modified Bligh and Dyer (1959) method. The amount of lipids in the chloroform extracts from all the samples were measured as total phosphovanillin positive materials (Frings *et al.*, 1972). Haemolymph samples from *in vivo* experiments were directly used for lipid estimation by the above method. The quantity of lipids was determined by comparing the values with those obtained by using glycerol trioleate as the standard. From the values obtained, changes in the pattern of lipid release due to the hormone action have been estimated.

Quantitation of proteins

Haemolymph sample (10 μl) collected before and after 30 min of AKH-I (100 pmoles) injection were diluted with invertebrate saline (0.67% NaCl, 25 μl) and centrifuged at 8500 $\times$ g for 5 min in a Beckman microfuge to precipitate the haemocytes and tissue fragments. An aliquot (5 μl) each were drawn from both control and experimental for quantitation of proteins by Bradford (1976) method to ascertain the quantity of protein in the sample used for electrophoresis.

Electrophoresis

Sample buffer (25 μl each) were added to the remaining portions of the supernatants described above, and warmed for 1 min in a boiling water bath. Aliquots (volume in the experimental and the control adjusted so as to load equal quantity of protein in each well) from these samples were applied in to the wells of SDS polyacrylamide gel plates for protein separation (according to a modified version of the method described by Laemmli (1970)) together with a third sample of mixture of protein standards. Two different concentrations of gels—spacer gel (3%) and separating gel (10%)—were used for the separation of proteins. Electrophoresis of the samples was carried out under a current of 100 V at a room temperature of about 20 °C. After running was completed, the gel was fixed in 50% methanol containing 50 μl formalin. After overnight fixing, the gel was washed in distilled water and stained with Coomassie brilliant blue. Staining and destaining were done for appropriate time periods and the relative electrophoretic mobility (R_m) of different protein bands were compared with those of the protein markers. Densitometric analysis of the protein tracks on the gel of the control and experimental samples were made using a gel scanner. The absorbance values of protein bands were set on the y-axis and their corresponding R_m values (represented as $R_m \times 100$) on the x-axis.

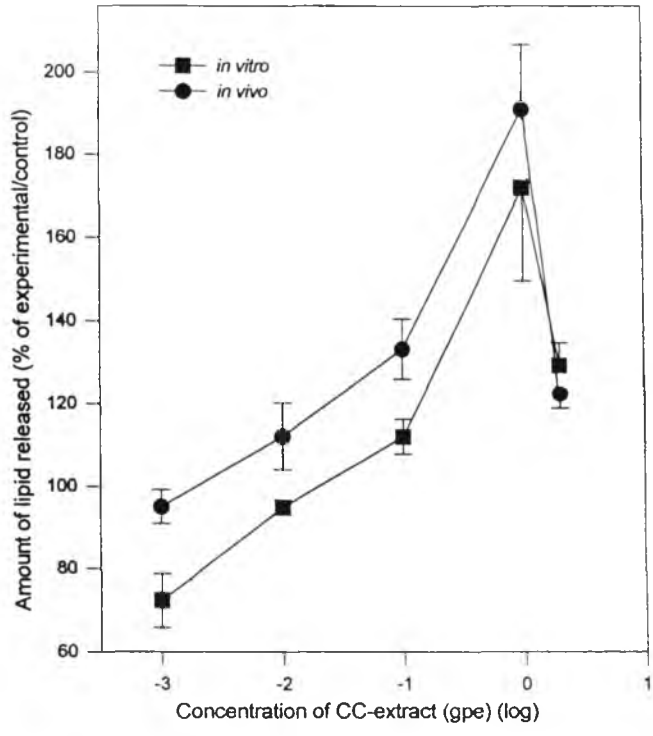

FIGURE 1. Effect of different concentrations of CC-extract on lipid release from the fat body of *I. limbata in vivo* and *in vitro*.

RESULTS

Effect of hormones on lipid release from the fat body *in vivo* and *in vitro*

Measurement of lipids released from the fat body of *I. limbata* incubated with various concentrations of the synthetic hormone and the extract *in vitro* and injected with different quantities of the hormones *in vivo* indicate that the hormones have dose-dependent adipokinetic effects on the fat body (Figs 1 and 2). The adipokinetic effect with the highest concentration, however, showed a downward trend in all the cases. Figure 1 shows the effect of the CC-extract on lipid release from the fat body *in vitro* and *in vivo*. The activity appeared to be better *in vivo* with respect to the sensitivity and extent of stimulation. A concentration of 0.01 gpe was sufficient to bring hyperlipaemia (increase 12%) in the haemolymph. A maximum of 91% stimulation of lipid release occurred at 1.0 gpe concentration. Further increase in concentration (2.0 gpe) showed a reduction in the extent of stimulation (to 23%). *In vitro* lipid release also showed a similar pattern of activity although the sensitivity and extent of release was slightly lower. Hyperlipaemic effect was initiated only with 0.1 gpe concentration of the extract. Maximum stimulation of lipid release obtained in this case was only 72% with 1.0 gpe concentration of the extract. Higher concentration

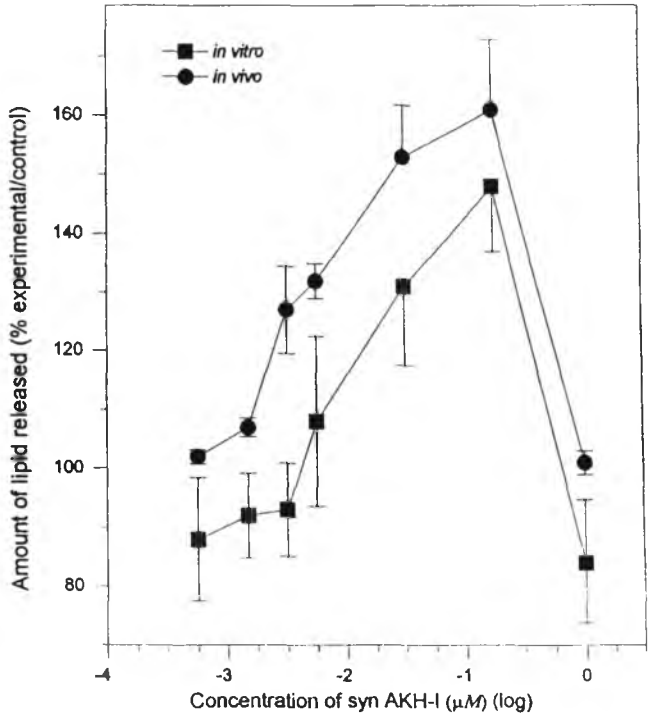

FIGURE 2. Effect of different concentrations of syn AKH-I on lipid release from the fat body of *I. limbata in vivo* and *in vitro*.

of the extract (2.0 gpe) reduced the extent of stimulation of lipid release (to 29%). Synthetic hormone showed more or less similar results as that obtained with the extract. However, the extent of stimulation by syn hormone was lower both *in vitro* and *in vivo*. Maximum stimulation of 61% was obtained *in vivo* with 0.5 μM concentration of the hormone whereas the maximum obtained *in vitro* was only 48% with 0.5 μM (Fig. 2). The maximum stimulation of lipid release from the fat body by syn AKH was considerably lower than that caused by the extract. With both the hormones, lipid release was found to be maximum *in vivo*. Likewise, maximum activity with hormone extract was obtained with 1.0 gpe and with the syn AKH-I it was with 0.5 μM.

Effect of AKH injection on haemolymph proteins

Examination of the stained gels (Fig. 3) and the densitograms (Figs 4 and 5) of the haemolymph proteins and lipoproteins from the controls and experimentals revealed that the injection of synthetic AKH-I into *I. limbata* resulted in qualitative as well as quantitative changes in the various proteins in the haemolymph. An additional band formed in the experimental in the high molecular weight region had an absorption peak at 0.39 (R_m 0.033). The absorbance (0.38) of the peak with R_m 0.32 in the

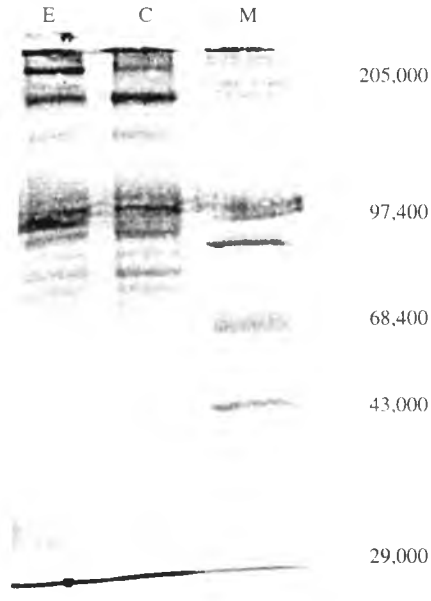

FIGURE 3. Haemolymph protein pattern of *I. limbata* after SDS-PAGE, E - Experimental (AKH-I injected); C - Control; M - Molecular markers.

control decreased (to 0.35) whereas that of a peak with absorbance 0.34 (R_m 0.39) has increased to 0.36. Another peak at R_m 0.42 shows a significant reduction in absorbance from 0.34 to 0.29. In the lower molecular weigh region, a few narrow peaks have consolidated into three wider peaks (R_m values 0.69, 0.85 and 0.9 respectively) with absorbance values in the range of 0.24 and 0.25. In brief, the results from electrophoresis and subsequent scanning of the gel indicate that lipid release from the fat body resulting from AKH-I injection have been accompanied by transformations of proteins and lipoproteins in the haemolymph. The experiments thus suggest that the neuronal tissues of *I. limbata* contain factors, which can elevate the level of lipids in the haemolymph accompanied by a change in the pattern of proteins and lipoproteins. It is seen that the injection of syn AKH-I into *Iphita* had resulted in the formation of a few new proteins and an increase in the quantity of proteins.

DISCUSSION

Results of experiments with native hormones extracted from the brain and associated glands (CC-CA complex) of *I. limbata* when compared with the results obtained from experiments with syn AKH-I, give strong indication that there are some adipokinetic factors contained in the neuronal tissues of the insect. The dose-dependent adipokinetic activity of the extract was found to be similar to such an

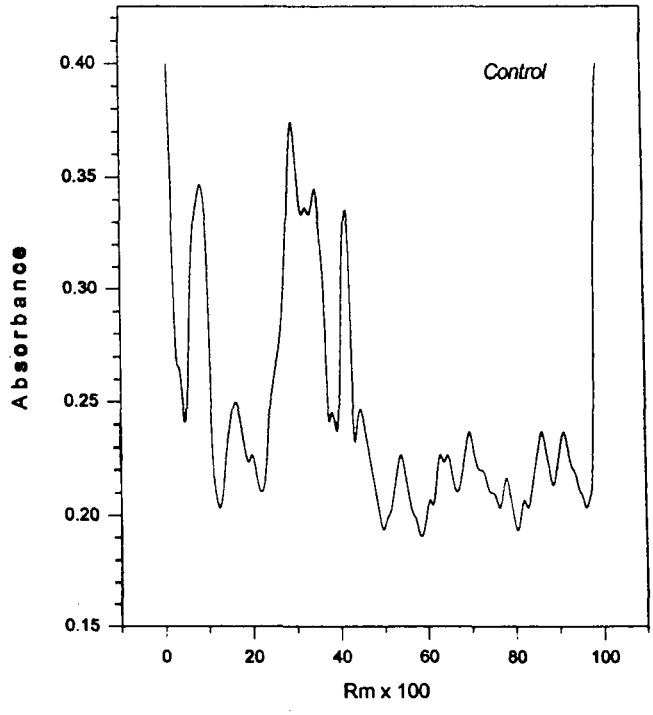

FIGURE 4. Densitogram of proteins from the haemolymph of *I. limbata* separated on SDS-PAGE (see the materials and methods section).

effect of syn AKH-I on the fat body. Dose dependent increase in haemolymph lipids in response to injection of CC-extract has been observed in the locust, *S. gregaria* (Mayer and Candy, 1969) which was similar to the activity of adipokinetic peptide hormones in vertebrates (Rudman, 1963). The similarity in activity of the extract in our experiment, to that of the synthetic hormone give an indirect indication as to the similarity of the active component in the extract to that of syn AKH-I. Although locust CC-extract has been found to be very potent in locust itself (Goldsworthy *et al.*, 1986), the activity of the hormone in *Iphita* has not been that much better. In *Iphita*, the native hormone stimulated lipid release by 91% (*in vivo*) although this required a concentration of 1.0 gpe (Fig. 1) which is very high compared to the effect of native hormone extracts in locusts. In the locusts, *L. migratoria* and *S. gregaria*, a saturation dose of the CC-extract was found to be 0.02 gpe, which elevated haemolymph lipid levels by 3 to 4 folds. However, in *Iphita*, syn Lom AKH-I elicited a maximum of only 61% increase in lipid release *in vivo*. This lower activity may be either due to the difference in the amino acid sequence of syn AKH-I and the active factor in *Iphita* or may be due to the difference in the binding property of receptors from species to species even if they possess the basic AKH-RPCH peptide sequence. Changes in the amino acid sequence of a peptide, whether or not these changes are conservative, have

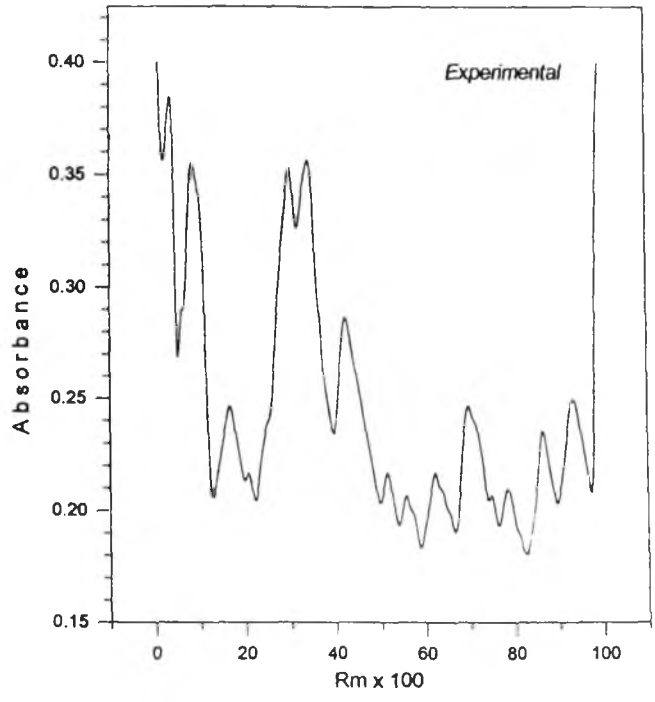

FIGURE 5. Densitogram of proteins from the haemolymph of *I. limbata* injected with syn AKH and separated on SDS-PAGE (see materials and methods section).

been found to reduce the activity as the structure decides the binding property of the peptide to the membrane receptors. Different binding properties have been detected for the cell membrane receptors in the fat body of the locusts, *L. migratoria* and *S. gregaria* (Stone *et al.*, 1978; Gaede, 1990).

The presence of adipokinetic factors in the neuronal tissues of *I. limbata* has further been supported by the results obtained by SDS-PAGE studies on the effect of hormone on the protein and lipoprotein pattern in the haemolymph. Since the activity of the extract was found to be of similar pattern to that of the syn hormone, syn hormone was used for injection for the initiation of lipoprotein transformation. The results obtained shows that some of the low molecular weight proteins transforms into relatively high molecular weight proteins. This is in agreement with the results obtained from other insects such as locusts where injection of AKH or release of hormone as a result of flight, triggers the interconversions of lipoproteins in the haemolymph (Wheeler, 1981; Chino *et al.*, 1986). The formation of A^{+}, the high molecular weight (low density) lipoproteins from A_{yellow} and C_L proteins (apoLp-III) (low molecular weight proteins) is followed by a decrease in the concentration of the latter ones, in the haemolymph of *Locusta migratoria migratorioides* (Wheeler and Goldsworthy, 1983). Lipoprotein conversions have been studied in other insects such as *Acherontia*

atropos (Surholt *et al.*, 1988) and *M. sexta* (Van Heusden and Law, 1989). It has been found that the concentration of C_L proteins in the haemolymph have a feed back inhibitory effect on the lipase activity which is reactivated when the concentration of C_L protein is decreased when lipoprotein A^+ is formed (Wheeler *et al.*, 1986).

Earlier, adipokinetic activity and the concomitant changes in the lipoprotein pattern were believed to be associated with flight. However, the presence and activity of such hormones were detected in non-flying insects such as the grasshopper, *Romalea microptera* (Spring and Gaede, 1987), and younger stages of various insects such as fifth instar locusts (Van der Horst *et al.*, 1987; Gokuldas *et al.*, 1988) and larval honey bee *Apis mellifera* (Robbs *et al.*, 1985). It is now realised that the hormone is not involved only in adult activities such as flight, but also in other activities where lipid mobilisation and utilisation is required.

The present studies also revealed that the hormone had better activity *in vivo* than *in vitro* which may be due to the presence of essential complements including lipoproteins present in the haemolymph, required for the smooth functioning of the lipid shuttle system. The observed drop in the stimulatory effect of higher concentration of both the extract and syn hormone *in vivo* and *in vitro* may be due to the feed back inhibition. It can also be due the presence of an increased titre of some counteracting (hypoglycaemic) factors, likely to be present in the crude extract. The fact that this drop is seen in the case of the syn hormone also, suggests the possibility of the presence of receptors on the fat body that get stimulated which at a higher density initiates some hypolipaemic activity. Experiments with the purified fractions of the extract will clarify whether it contains any counteracting factors. Likewise, SDS-PAGE of purified lipoproteins or specific staining for lipoproteins would help to identify more specifically the pattern of transformation of lipoproteins.

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REFERENCES

Asher, C., Moshitzky, P., Ramachandran, J. and Applebaum, S. W. (1984) The effects of synthetic locust adipokinetic hormone on dispersed locust fat body cell preparation: cyclic AMP induction, lipid mobilization and inhibition of protein synthesis, *Gen. Comp. Endocrinol.* **55**: 167–173.

Beenakkers, A. M. Th. (1969) The influence of corpus cardiacum on lipid metabolism in *Locusta migratoria*, *Gen. Comp. Endocrinol.* **13**: 492.

Beenakkers, A. M. Th., Van der Horst, D. J. and Van Marrewijk, W. J. A. (1981) Metabolism during locust flight, *Comp. Biochem. Physiol.* **69**: 315–321.

Beenakkers, A. M. Th., Van der Horst, D. J. and Van Marrewijk, W. J. A. (1985) Biochemical processes directed to flight muscle metabolism, In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Vol. 10. Kerkut, G. A. and Gilbert, L. I. (Editors). Pergamon Press: Oxford, **10**: 451–486.

Bligh, E. G. and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification, *Can. J. Biochem. Physiol.* **37**: 911–917.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein dye-binding, *Analyt. Biochem.* **72**: 248–254.

Carlisle, J. A. and Loughton, B. G. (1979) Adipokinetic hormone inhibits protein synthesis in *Locusta*, *Nature* **282**: 420–421.

Carlisle, J. A. and Loughton, B. G. (1986) The inhibition of protein synthesis *in Locusta migratoria* by adipokinetic hormone, *J. Insect Physiol.* **32**: 573–578.

Chino, H., Downer, R. G. H. and Takahashi, K. (1986) Effect of adipokinetic hormone on the structure and properties of lipophorin in locusts, *J. lipid Res.* **27**: 21–29.

Frings, C. S., Fendly, T. W., Dunn, R. J. and Queen, C. A. (1972) Improved determination of total serum lipids by the sulfo-phosphovanillin reaction, *Clin. Chem.* **18**: 673–674.

Gaede, G. (1990) The adipokinetic hormone/red pigment-concentrating hormone peptide family: structures, interrelationships and functions, *J. Insect Physiol.* **36**: 1.

Gaede, G. (1991) The adipokinetic neuropeptide of Mantoidea: sequence elucidation and evolutionary relationships, *Biol. Chem. Hoppe-Seyler* **372**: 193–201.

Gaede, G. (1992) Insect neuropeptides, In: *Advances in Regulation of Insect Reproduction*, Bennettova, B., Gelbic, I and Soldan, T. (Editors). Institute of Entomology, Czech. Acad. Sci.: Czeske, Budejovice, 67–80.

Gokuldas, M., Hunt, P. A. and Candy, D. J. (1988) The inhibition of lipid synthesis *in vitro* in the Locust, *Schistocerca gregaria* by factors from the corpora cardiaca, *Physiol. Entomol.* **13**: 43–48.

Goldsworthy, G. J. and Wheeler, C. H. (1984) Adipokinetic hormones in locusts, In: *Biosynthesis, Metabolism and Action of Invertebrate Hormones*, Hoffmann, J. A. and Porchet, M. (Editors). Springer: Berlin, 126–135.

Goldsworthy, G. J., Mallison, K. and Wheeler, C. H. (1986) The relative potencies of two known locust adipokinetic hormones, *J. Insect Physiol.* **32**: 95–101.

Jaffe, H., Raina, A. K., Riley, C. T., Fraser, B. A., Bird, T. G., Tseng, C., Zang, Y and Hayes, D. K. (1988) Isolation and primary structure of a neuropeptide hormone from *Heliothis zea* with hypertrehalosemic and adipokinetic activities, *Biochem. Biophys. Res. Comm.* **155**: 344–350.

Kodrik, D. and Goldsworthy, G. J. (1994) Adipokinetic hormones inhibit RNA synthesis in the fat body of adult *Locusta migratoria*, In: *Neurochemistry and Neurophysiology*, Borkovec, A. B. and Loeb, M. J. (Editors). CRC Press: Boca Raton, 173–176.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature* **227**: 680–685.

Mayer, R. J. and Candy, D. J. (1969) Control of haemolymph lipid concentration during locust flight. An adipokinetic hormone from the corpora cardiaca, *J. Insect Physiol.* **15**: 611–620.

Robbs, S. L., Ryan, R. O., Schmidt, J. O., Keim, P. S. and Law, J. H. (1985) Lipophorin of the larval honeybee, *Apis mellifera*, *J. lipid Res.* **26**: 241–247.

Robinson, N. L. and Goldsworthy, G. J. (1977) Adipokinetic hormone and the regulation of carbohydrate metabolism in a working flight muscle preparation, *J. Insect Physiol.* **23**: 9–16.

Rudman, D. (1963) The adipokinetic action of polypeptide and amine hormones upon the adipose tissue of various animal species, *J. Lipid Res.* **4**: 119–129.

Shapiro, J. P., Law, J. H. and Wells, M. A. (1988) Lipid transport in insects, *Ann. Rev. Entomol.* **33**: 297–318.

Spring, J. H. and Gaede, G. (1987) Factors regulating carbohydrate and lipid metabolism isolated from the corpus cardiacum of the eastern luber grasshopper, *Romalea microptera*, *J. Exp. Zool.* **241**: 41–50.

Stone, J. V., Mordue, W., Broomfield, C. E. and Hardy, P. M. (1978) Structure-activity relationship for the lipid mobilising action of locust AKH: synthesis and activity of a series of hormone analogues, *Eur. J. Biochem.* **89**: 195–202.

Surholt, B., Thomas, K. F., Goldberg, S. J., Dick, J., Van der Horst, A. M. Th. and Beenakkers, (1988) Lipophorin conversion during flight of the deaths–head hawk Moth *Acherontia atropos*, *Insect Biochem.* **18**: 117–126.

Van Antwerpan, R., Linnemans, W. A. M., Van der Horst, D. J. and Beenakkers, A. M. Th. (1988) Immunocytochemical localisation of lipophorins in the flight muscles of the migratory locust (*Locusta migratoria*) at rest and during flight, *Cell Tiss. Res.* **252**: 661–666.

Van der Horst, D. J. (1983) Lipid transport in insects, In: *Metabolic Aspects of Lipidf Nutrition in Insects*, Mittler, T. E. and Dadd, R. H. (Editors). Westriew Press: Boulder, Colarado, 183–202.

Van der Horst, D. J., Beenakkers, A. M. Th., Van Doorn, J. M., Gerritse, K. and Schultz, T. K. F. (1987) Adipokinetic hormone-induced lipid mobilisation and lipophorin interconversions in locusts, *Insect Biochem* **17**: 799–808.

Van Heusden, M. C. and Law, J. H. (1989) An insect lipid transfer particle promotes lipid loading from fat body to lipoprotein, *J. Biol. Chem.* **264**: 17287–17292.

Wheeler, C. H. (1981) Haemolymph proteins of several insects, *J. Insect Physiol.* **14**: 1069–1075.

Wheeler, C. H. and Goldsworthy, G. J. (1983) Qualitative and quantitative changes in *Locusta* haemolymph proteins and lipoproteins during ageing and adipokinetic hormone action, *J. Insect Physiol.* **29**: 339–347.

Wheeler, C. H., Bothby, K. M. and Goldsworthy, G. J. (1986) C_L-proteins and the regulation of lipoprotein lipase activity in locust flight muscle, *Biol. Chem. Hoppe-Seyler* **367**: 1127–1133.

Woodring, J. and Leprince, D. J (1992) The function of corpus cardiacum peptides in horseflies, *J. Insect Physiol.* **38**: 775–782.

Ziegler, R. (1979) Hyperglycaemic factor from the corpora cardiaca of *Manduca sexta* (L) (Lepidoptera: Sphingidae), *Gen. Comp. Endocrinol.* **39**: 350–357.

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Seasonal activity of stem borers and their natural enemies on fodder maize

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ABSTRACT: Percent infestation of borers, larval population, percent larval disease and pupal parasitism differed significantly between years and seasons of planting of fodder maize. Mean percent infestation during *kharif* (35.4%) was significantly higher than in other seasons in both years. Similarly larval population and their natural enemies and pupal parasitism was significantly more in *kharif* and late summer seasons compared to late *kharif*, *rabi* and summer seasons. However mean pupal population, larval parasitism and predators did not differ significantly between the years. *Chilo partellus* (Swinhoe) was the dominant borer (90–95 percent). *Sesamia inferens* (Walker) was recorded more during *rabi* season. A total of 42 natural enemies were recorded on *C. partellus*. Among various natural enemies recorded on *C. partellus*, *Cotesia flavipes* (Cameron) in larval stage and *Xanthopimpla stemmator* (Thunberg) and *Tetrastichus howardi* (Olliff) in pupal stage were most important and frequently collected. Larval predatory population consisted mainly of anthocorid bug, *Orius tantillus* Motschulsky, two species of reduviid bugs and seventeen species of spiders. In pupal stage the only predator recorded was earwig, *Euborellia annulipes* (Lucas). In the present investigation seven larval parasitoids, 17 predators and NPV were recorded for the first time in India.

KEYWORDS: Fodder maize, natural enemies, *Chilo partellus*, *Sesamia inferens*, seasonal activity

INTRODUCTION

The spotted stem borer *Chilo partellus* (Swinhoe) is the most important pest of maize and sorghum all over the country and in many parts of Asia and Africa. The importance of the pest can be assessed from the yield reduction that it can cause. Chatterji *et al.* (1969) reported upto 36% loss of grain maize in Delhi, while Gupta (1972) reported heavy infestation in fodder maize in Haryana. In sorghum, loss is recorded even up to 88% (Seshu Reddy, 1988). In Punjab, Singh *et al.* (1975) observed that *C. partellus*

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breeds in summer and late summer of fodder maize and then migrates to grain maize during *kharif* season to cause severe economic loss.

One of the most important features in development of bio-intensive integrated pest management for *C. partellus* is to know the population changes and abundance of natural enemies throughout the year. Though few studies were conducted on seasonal changes of the population in grain maize in the country especially, from March to November, similar information of fodder maize or the effect and abundance of natural enemies on population fluctuation throughout the year were lacking. The studies were, therefore, carried out for 2 years to determine seasonal fluctuations in borer population and their natural enemies on fodder maize ecosystem in Bangalore.

MATERIALS AND METHODS

Studies were conducted in Bangalore district from June 1994 to May 1996. Observations were recorded at weekly interval in seven randomly selected spots each plot measuring (6 × 4 m) in a hectare adopting destructive sampling technique. Out of these, four spots was randomly selected on the outer four sides and three in the inner side of the field. In each of these spots 50 plants were observed randomly for pest incidence and a total of 350 plants were observed once in a week. The leaves/plants showing 'shot hole'/'deadhearts' symptoms were considered as infested by the borers. From each spot all the infested plants were removed for observing number of eggs, larvae and pupae of the borers. All immature stages were reared in the laboratory for obtaining parasitoids and recording disease. Predators were counted in the field itself on infested plants except those that were inside the whorls or the stem, which were counted in the laboratory. Larval disease was determined in each sample after observing under the microscope in the laboratory. Observations on each of crops were recorded from borer infestation to harvest.

From the data recorded, percent incidence of two borers, i.e. *C. partellus* and *Sesamia inferens* (Walker) were derived by counting total number of larvae and each species collected. The number of *C. partellus* and *S. inferens* larvae and percentage of each borer was worked out on each infested plant. The number of larval instar in field sample was worked out based on Dyar (1890).

Weekly data on infestation, larval and pupal population and their parasitoids, predators and entomopathogens was pooled in the each season and were subjected to arcsine transformation before analysis for comparing differences in different seasons. The data of both years was subject to two-way ANOVA and means were separated by LSD values. The mean maximum temperature, minimum temperature and RH during *kharif*, late *kharif*, *rabi*, summer and late summer seasons were 27.2°, 18.2 °C and 76%; 25.8°, 15.7 °C and 65%; 27.5°, 16.3 °C and 52%; 32.6°, 21.0 °C and 60 and 29.1°, 19.2 °C and 78%, respectively.

RESULTS AND DISCUSSION

The weekly observations recorded during different seasons on the percent plants infested by borers, larval and pupal population, percent larval and pupal parasitism, percent larval disease and predator numbers for the years 1994–96.

Borers

The highest mean percent infestation of 39.1 percent (range 5.6–56.8 percent) in 1st year and 31.7 percent (range 18.5–33.0 percent) was recorded in 2nd year in *kharif* and it differed significantly between the years and with other seasons (Table 1). The infestation range recorded in late *kharif*, *rabi*, summer and late summer seasons was 0.0–23.9, 5.4–10.1; 3.9–17.6, 4.5–6.2; 4.2–8.8, 5.3–29.3; 8.2–44.8 percent for 1st and 2nd years, respectively. Data recorded for 2 years showed that infestation starts building up in late summer season thus resulting in high infestation during ensuing *kharif* season. Infestation on fodder maize followed a set patter, it started 1–2 weeks after crop germination in all the seasons except in *rabi*, when it occurred from 3rd week after crop germination. Oloo (1989) also reported that *C. partellus* infestation starts from 2nd week after crop germination in Kenya. *C. partellus* was observed to be a dominant species. Percentage of *C. partellus* larval population ranged from 89.8–100.0 and that of *S. inferens* from 0.0–11.5 percent in season except in *rabi* season, when *S. inferens* population ranged from 0.6–24.4 percent. Kfir (1992) observed *C. partellus* to be dominant stem borer in South Africa. *Sesamia inferens* was recorded attacking young plants. Present study on its high availability during *rabi* season agrees with the findings of Rajagopal and ChannaBasavanna (1975). In earlier study in Punjab (from March to October 1974), *C. partellus* infestation and larval activity was also reported to be more during late summer and *kharif* months (Singh *et al.*, 1975) as observed in the present study. In the *kharif* season temperature ranges from 20–30 °C in the field and this provides optimum temperature for *C. partellus* to breed and become serious pest. These observations were confirmed by the laboratory study where highest fecundity, survival and shortest larval period of the pest were recorded at this temperature range.

The mean larval population per infested plant was significantly more during *kharif* season (4.5 and 4.0/infested plants) for 1st and 2nd year and it differed significantly between the years and between season (LSD, $P = 0.05$) (Table 1). The larval instar distribution in the field revealed that in *kharif*, late *kharif* and late summer seasons 6th instar larvae were available by 5th week, whereas in rabi by 8th week and in summer by 4th week after infestation. Thus, larval period was extended by 7–10 days in *rabi* and was shortened by a week during summer season. The larval population of *C. partellus* was generally high at the whorl stage of the crop in each season.

The mean pupal population per infested plant was significantly higher in *kharif* season 0.87 and 0.57/infested plants in both years and it varied significantly between the seasons (Table 1). However, no significant difference was observed in pupal population between the years. The availability of pupae in all seasons indicated that *C. partellus* could survive throughout the year.

TABLE 1. Seasonal activity of *C. partellus* on fodder maize at Bangalore during 1994–95 (1st year) and 1995–96 (2nd year)

Season	Percent infestation*[1]			Larval population/infested plant*			Pupal population/infested plant*[1]		
	1st year	2nd year	Season mean	1st year	2nd year	Season mean	1st year	2nd year	Season mean
Kharif	39.1 (38.6)	31.7 (34.3)	35.4[a] (36.5)	4.50	4.00	4.25[a]	0.87	0.57	0.72[a]
Late *kharif*	21.8 (27.8)	15.8 (23.3)	18.8[c] (25.7)	2.60	2.60	2.60[c]	0.32	0.39	0.36[c]
Rabi	15.8 (23.5)	12.8 (20.9)	14.3[d] (22.2)	2.00	2.60	2.30[d]	0.39	0.41	0.40[c]
Summer	14.1 (21.9)	25.1 (30.1)	19.6[b] (26.2)	2.40	3.20	2.80[c]	0.30	0.55	0.43[b]
Late summer	30.7 (33.6)	— (—)	30.7[b] (33.6)	3.40	—	3.40[b]	0.48	—	0.48[b]
Year mean	24.3[a] (29.6)	21.4[b] (27.4)		2.98[b]	3.10[a]	0.47	0.48		
	A factor	B factor	A × B	A factor	B factor	A × B	A factor	B factor	A × B
SEM	0.16	0.23	0.31	0.05	0.09	0.14	0.01	0.01	0.02
LSD ($P = 0.05$)	0.44	0.61	0.89	0.11	0.26	0.41	NS	0.03	0.04

*Values followed by the same letter in the same column are not significantly differed (LSD, $P = 0.05$). [1]Figures in parenthesis are arcsine transformed values. A factor = year, B factor = season, A × B = interaction between season × year.

Natural enemies

Parasitoids

C. partellus larval parasitism was at par for *kharif* and late summer seasons with mean parasitism of 26.5 (range 0.0–45.3 percent) and 26.6 percent (range 7.7–32.4 percent), respectively and it differed significantly between other seasons (LSD 0.73, $P = 0.05$). The range of parasitism recorded in late *kharif*, *rabi* and summer seasons 0.0–21.4, 0.0–25.0 and 0.0–26.4 percent, respectively.

The pupal parasitism was more during 1st year (20.6–28.7 percent) compared to 2nd year (14.3–30.6 percent) (LSD 0.68, $P = 0.05$) and in *kharif* it was significantly higher than other season in both years (LSD 1.08, $P = 0.05$) (Table 2). The pupal parasitism range obtained in *kharif* was 7.1–55.0, in late *kharif* 0.0–33.4, in *rabi* 0.0–36.0, in summer 4.0–33.3 and in late summer it ranged from 0.0–37.5 percent.

A total of 42 natural enemies were recorded the study period. The parasitoids recorded on egg stage was *Trichogramma chilonis* Ishii, which was present in all seasons. On larval stage parasitoids recorded were *Cotesia flavipes* (Cameron), *Myosoma chinensis* (Szépligeti), *Stenobracon nicevillei* (Bingham), three species of *Bracon* sp. (first record), *Apanteles* sp., *Chelonus* sp., *Ichneumon* sp. (first record), *Sturmiopsis inferens* Townsend, *Argyrophylax* sp. *frasnsseni* (Baranov) (first record), *Argyrophylax* sp. nr. *apta* (Walker) (first record), *Sysropa* sp. *picta* (Baranov) (first record). Amongst larval parasitoids only *C. flavipes* was available throughout the year. The pupal parasitoids recorded were *Brachymeria nosatoi* Habu (first record), *Tetrastichus howardi* (Olliff) and *Xanthopimpla stemmator* (Thunberg), latter two parasitoids were available in all the seasons (Table 3). In the present study, seven species of larval parasitoids and one pupal parasitoid was recorded for the first time in India on *C. partellus*.

Predators

There was non-significant difference in predatory population between the years but it differed significantly between the seasons in both years (LSD 0.11, $P = 0.05$). In *kharif*, predatory population (mean 2.1/infested plant) was significantly higher compared to other seasons (Table 2). The predatory population ranged from 0.5–4.3, 0.0–2.3, 0.3–2.8, 0.0–0.9 and 0.4–2.3/infested plant in *kharif*, late *kharif*, *rabi*, summer and late summer, respectively. The predators recorded were two anthocorides—*Orius tantillus* Motschulsky (first record) and *Blaptostethsus pallescens* Poppius (first record); two reduviids—*Acanthaspis* sp. and *Coranus spiniscutis* Reuter (first record); seventeen species of spiders—*Argiope pulchella* Thorell, *Neoscona* sp., *N. bengalensis* Thorell, *N. mukerjei* Tikader, *Cheiracanthium melanostoma* Thorell, *Clubiona drassodes* Cambridge, *C. Iudhianensis* Tikader, *Heteropoda* sp., *Olios iranii* (Pocock), *Pardosa* sp., *Oxyopes sakuntalae* Tikader, *O. shweta* Tikader, *Hentzia* sp., *Marpissa* sp., *Phidippus* sp., *P. pateli* Tikader and *Thomissus* sp. and in pupal stage predator recorded was *Euborellia annulipes* (Lucas). Amongst predator *O. tantallius*, both reduviid bugs and 12 species of spiders were present in all seasons (Table 3).

TABLE 2. Seasonal activity of natural enemies of *C. partellus* on fodder maize at Bangalore during 1994–95 (1st year) and 1995–96 (2nd year)

Season	Percent larval parasitism*[1]			Percent pupal parasitism*[1]			Predators/infested plant*			Percent diseased larvae*[1]		
	1st year	2nd year	Season mean	1st year	2nd year	Season mean	1st year	2nd year	Season mean	1st year	2nd year	Season mean
Kharif	27.5	25.5	26.5[a]	28.7	30.6	29.6[a]	2.50	1.70	2.10[a]	21.5	19.2	20.3[a]
	(31.6)	(30.3)	(30.9)	(32.4)	(33.6)	(32.9)				(27.6)	(26.0)	(26.8)
Late *kharif*	13.3	19.3	16.3[c]	20.6	22.3	21.5[d]	0.92	1.30	1.11[c]	18.1	18.1	18.1[b]
	(21.4)	(26.1)	(23.8)	(27.0)	(28.2)	(27.6)				(25.2)	(25.2)	(25.2)
Rabi	17.0	13.2	15.1[d]	27.2	14.3	20.8[d]	1.00	1.10	1.05[c]	14.5	22.0	18.3[b]
	(24.3)	(21.3)	(22.8)	(31.4)	(22.2)	(27.1)				(22.4)	(27.9)	(23.3)
Summer	16.8	22.5	19.7[b]	23.8	26.0	24.9[c]	0.60	0.80	0.70[d]	16.2	15.6	15.9[c]
	(24.2)	(28.3)	(26.3)	(29.2)	(30.6)	(29.9)				(23.7)	(23.3)	(26.5)
Late summer	26.6	—	26.6[a]	27.2	—	27.2[b]	1.50	—	1.50[b]	19.3	—	19.3
	(31.0)	—	(31.0)	(31.4)	—	(31.4)	—			(26.1)	—	(26.1)
Year mean	20.2	20.1		25.5[a]	23.3[b]		1.55	1.23		17.9[b]	18.7[a]	
	(26.7)	(26.6)		(30.3)	(28.9)					(25.0)	(25.6)	
	A factor	B factor	A × B	A factor	B factor	A × B	A factor	B factor	A × B	A factor	B factor	A × B
SEM	0.05	0.25	0.34	0.21	0.37	0.57	0.07	0.04	0.07	0.14	0.22	0.30
LSD ($P = 0.05$)	NS	0.73	0.93	0.68	1.08	1.54	NS	0.11	0.21	0.41	0.63	0.86

*Values followed by the same letter in the same column are not significantly differed (LSD, $P = 0.05$). [1]Figures in parenthesis are arcsine transformed values. A factor = year, B factor = season, A × B = interaction between season × year.

TABLE 3. Natural enemies of *C. partellus* on fodder maize ecosystem

Sl. No.	Natural enemy	Stage attacked	Order : Family	Seasonality
	Parasitoids			
	Egg parasitoid			
1.	*Trichogramma chilonis* Ishii	Egg	Hymenoptera: Trichogrammatidae	All
	Larval parasitoids			
2.	*Cotesia flavipes* (Cameron)	Larval	Hymenoptera: Braconidae	All
3.	*Mysoma chinenesis* (Szépligeti)	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*, late summer
4.	*Stenobracon nicevillei (Bingham)*	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*, late summer
5.	*Bracon* sp. 1*	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*, late summer
6.	*Bracon* sp. 2*	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*
7.	*Bracon* sp. 3*	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*
8.	*Apanteles* sp.	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*
9.	*Chelonus* sp.	Larval	Hymenoptera: Braconidae	*Kharif*, late *kharif*, *rabi*
10.	*Ichneumon* sp.*	Larval	Hymenoptera: Ichneumonidae	*Kharif*, late *kharif*, *rabi*
11.	*Sturmiopsis inferens* Townsend	Larval	Diptera: Tachinidae	Late *kharif*, *rabi*
12.	*Argyrophylax* sp. *fransesni* (Baranov)*	Larval	Diptera: Tachinidae	*Kharif*
13.	*Argyrophylax* sp. nr. *apta* (Walker)*	Larval	Diptera: Tachinidae	*Kharif*
14.	*Sysropa* sp. *picta* (Baranov)*	Larval	Diptera: Tachinidae	*Kharif*
	Potential larval predator			
15.	*Orius tantillus* Motschulsky*	Larval	Heteroptera: Anthocoridae	All
16.	*Blaptostethus pallescens* Poppius*	Larval	Heteroptera: Anthocoridae	*Kharif*, late summer
17.	*Acanthaspis* sp.	Larval	Heteroptera: Reduviide	All
18.	*Coranus spiniscutis* Reuter*	Larval	Heteroptera: Reduviide	All
19.	*Argiope pulchella* Thorell	Larval	Araneae: Araneidae	All
20.	*Neoscona* sp.	Larval	Araneae: Araneidae	All
21.	*N. bengalensis* Thorell	Larval	Araneae: Araneidae	*Kharif*
22.	*N. mukerjei* Tikader	Larval	Araneae: Araneidae	*Kharif*

TABLE 3. Continued ...

Sl. No.	Natural enemy	Stage attacked	Order : Family	Seasonality
23.	*Cheiracanthium melanostoma* Thorell	Larval	Araneae: Clubionidae	*Kharif*
24.	*Clubiona drassodes* Cambridge	Larval	Araneae: Clubionidae	*Kharif*
25.	*C. ludhianensis* Tikader	Larval	Araneae: Clubionidae	All
26.	*Heteropoda* sp.	Larval	Araneae: Heteropodidae	All
27.	*Olios iranii* (Pocock)	Larval	Araneae: Heteropodidae	*Kharif*
28.	*Pardosa* sp.	Larval	Aranae: Lycosidae	All
29.	*Oxyopes sakuntalae* Tikader	Larval	Araneae: Oxyopidae	*Kharif*
30.	*O. shweta* Tikader	Larval	Araneae: Oxyopidae	All
31.	*Hentzia* sp.	Larval	Arnaeae: Salticidae	*Kharif*
32.	*Marpissa* sp.	Larval	Arnaeae: Salticidae	All
33.	*Phidippus* sp.	Larval	Arnaeae: Salticidae	All
34.	*P. pateli* Tikader	Larval	Arnaeae: Salticidae	All
35.	*Thomissus* sp.	Larval	Arnaeae: Thomisidae	All
	Larval pathogens			
36.	*Nosema* sp.	Larval	Microspora: Nosemattidae	All
37.	Nuclear ployhedorsis virus	Larval	Virales: Baculoviridae	*Kharif*, late *kharif* and late summer
38.	*Beauveria bassiana* (Balsamo) Vuillemin	Larval	Monilales: Moniliaceae	*Kharif*, late *kharif* and late summer
	Pupal parasitoids			
39.	*Brachymeria nosatoi* Habu*	Pupal	Hymenoptera: Chalcididae	*Kharif*
40.	*Tetrastichus howardi* (Olliff)	Pupal	Hymenoptera: Eulophidae	All
41.	*Xanthopimpla stemmator* (Thunberg)	Pupal	Hymenoptera: Ichneumonidae	All
	Potential pupal predator			
42.	*Euboreilla annulipes* (Lucas)	Pupal	Dermaptera: Carcinophoridae	All

*First record on *C. partellus* in India.

Entomopathogens

The entomopathogen incidence was highest in *kharif* 20.3 percent (range 4.4–28.7 percent) and the differences observed between seasons were significant (LSD 0.63, $P = 0.05$) (Table 2). The percent larval disease ranged from 0.0–19.9, 1.6–33.2, 0.0–12.7 and 5.1–16.5 percent in late *kharif*, *rabi*, summer and late summer, respectively. Entomopathogens recorded were—*Nosema* sp., Nucleopolyhedrosis virus and *Beauveria bassiana* (Balsamo) Vuillemin. The record of NPV was for the first time in India (Table 3).

Natural enemies were recorded in higher numbers when high pest population was recorded during *kharif* season. In the present investigations, among various parasitoids, larval and pupal parasitoids were most important. Oloo (1989) in Kenya and Kfir (1992) in South Africa reported that the pupal parasitoids were more active and effective than egg or larval parasitoids on *C. partellus*. Microbial agents were also found to influence the larval population in some seasons. In northern parts of India, number of pathogens were reported on *C. partellus* (Mathur *et al.*, 1966; Atwal *et al.*, 1973; Sinha and Prasad, 1975). In Kenya, Odino *et al.* (1989) recorded the occurrence of number of pathogens affecting larvae and pupae of *C. partellus*.

In the present study for the first time in India quantification of borer infestation, larval and pupal population and also the role of natural enemies of *C. partellus* was done throughout the year.

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REFERENCES

Atwal, A. S., Singh, B. and Battu, G. S. (1973) *Chilo partellus* (Swinhoe) a new host of *Aspergillus flavus* Link and *Fusarium* sp., *Curr. Sci.* **42**: 585.

Chatterji, S. M., Young, W. R., Sharma, G. C., Sayi, I. V., Chahal, B. S., Khare, B. P., Rathore, Y. S., Panwar, V. P. S. and Siddiqui, K. H. (1969) Estimation of loss in yield of maize due to insect pests with special reference to borers, *Indian J. Ento.* **31**: 109–115.

Dyar, H. C. (1890) The number of moult of lepidopterous larvae, *Psyche* **5**: 42–422.

Gupta, J. C. (1972) Incidence of major insect pests of early summer fodders in Haryana, India, *F. A. O. Plant Prot. Bull.* **20**: 36–38.

Kfir, R. (1992) Seasonal abundance of the stem borer, *Chilo partellus* (Lepidoptera: Pyralidae) and its parasites on summer grain crops, *J. Econ. Entomol.* **85**: 518–529.

Mathur, S. B., Srivastava, R. P. and Subba Rao, B. R. (1966) *Beauveria densa* pathogenic for the larvae of *Chilo partellus*, *J. Inverte. Pathol.* **8**: 274–276.

Odindo, M. O., Otieno, W. A., Oloo, G. W., Kilori, J. I. and Odhiambo, R. C. (1989) Prevalence of micro organism in field-sampled borers on sorghum, maize and cowpea in western Kenya, *Insect Sci. Applic.* **10**: 225–228.

Oloo, G. W. (1989) The role of local natural enemies in population dynamics of *Chilo partellus* (Swinhoe) (Pyralidae) under subsistence farming systems in Kenya, *Insect. Sci. Applic.* **10**: 243–251.

Rajagopal, D. and ChannaBasavanna, G. P. (1975) Insect pests of maize in Karnataka, *Mysore J. Agri. Sci.* **9**: 110–121.

Seshu Reddy, K. V. (1988) Assessment of on-farm yield losses in sorghum due to insect pests, *Insect Sci. Applic.* **9**: 679–685.

Singh, B., Dhaliwal, J. S. and Atwal, A. S. (1975) Population studies on the maize borer, *Chilo partellus* (Swinhoe) in the Punjab. II. Population build-up during the active season, *Indian J. Ecol.* **2**: 23–29.

Sinha, S. K. and Prasad, M. (1975) A biological approach to the control of maize borer *Chilo zonellus* (Swinhoe), *Curr. Sci.* **44**: 197–198.

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Lipase activity in the fat body of *Chilo partellus* during larval growth and metamorphosis

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ABSTRACT: The maximum lipase activity was observed at pH 8 in 19-day old larval fat body and at pH 7.6 in 4-day old pupal fat body of *Chilo partellus*. Gradual increase in lipase activity in the larval fat body was observed from 8 to 11-day old larvae, sharp fall from 11 to 12-day old larvae, gradual increase from 12 to 20-day old larvae and decrease from 20 to 25-day old larvae. Maximum lipase activity was noted in 11-day old larvae and minimum in 12 and 25-day old larvae.

During metamorphosis, gradual increase in fat body lipase activity was noticed in prepupa (Po) to 2-day old pupae, sharp increase from 2 to 3-day old pupae and decline in the activity from 3 to 4-day old pupae. The sharp fall in enzyme activity was noticed in 4 to 5-day old male pupae and 4 to 6-day old female pupae and gradual decrease from 5 to 10-day old male pupae and 6 to 10-day old female pupae. Maximum fat body lipase activity was observed in 4-day old pupae and minimum in 10-day old pupae. The physiological role of the enzyme during larval growth and metamorphosis is discussed.

KEYWORDS: Lipase, fat body, larval growth, metamorphosis, *Chilo partellus*

INTRODUCTION

A few studies have been carried out on the activity of lipase in insect fat body which include lipase activity in the fat body of the desert locust *Schistocera gregaria* (George and Eapen, 1959b), diglyceride release from insect fat body (*H. cecropia*) a possible means of lipid transport (Chino and Gilbert, 1964), lipolytic activity in some tissues of *H. cercopia* and *P. americana* and its significance in lipid transport (Gilbert *et al.*, 1965), haemolymph lipid and fat body lipases of the Southern armyworm moth *Prodenia eridania*. (Stevenson, 1969), lipase activity in tissues of third instar larvae of the blowfly, *Calliphora erythrocephala* (Price, 1975), end product specificity of triacylglycerol lipase from intestine, fat body, muscle and haemolymph of the American cockroach, *Periplanata americana* L. (Hoffman and

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Downer, 1979), fatty acid composition of fat body and malpighian tubules of the Tenebrionid beetle, *Zophobas atratus*. Howard and Styanley-Samuelson (1996), lipase activity in the fat body and some tissues of *Chrysomyia rufifacies* during larval growth and metamorphosis (Pol and Sawant, 1997, 1999).

However the information on the fat body of *Chilo partellus* is rather scanty. There exist a lacuna in the field of lipase activity in fat body during larval growth and metamorphosis of *C. partellus* which is mainly concerned with release of energy, and supply of structural components of developing adult. Therefore, the present study attempts to provide information on lipase activity in the fat body of *C. partellus*, during larval growth and metamorphosis. The results are discussed with regards to the changes undergone during larval growth and metamorphosis.

MATERIAL AND METHODS

The culture of *Chilo partellus* was maintained in our laboratory on natural food of cut pieces of fresh maize stems. Hatching of eggs occurred within 6-days after oviposition. The larval growth was computed from the mean time of egg hatching (±1 hr) to the prepupal stage. The larval stage lasted for 25-days and metamorphosis stage for 10-days in the month of July, 2000. The studies were carried out at an interval of 24 hours. For the study of fat body lipase activity the following stages were selected during larval growth and metamorphosis i.e. 8 to 25-day larvae, prepupae to 10-day pupae.

All the solvents were of reagent grade and were obtained from E. Merk and Co. Rathway, N.J., U.S.A. and B.D.H., England. Unless otherwise indicated, solvents were redistilled in the laboratory under anhydrous condition before use. Diphenyl carbazid was purchased from E. Merk, Dermstat, Germany. Diphenyl Carbazone was of Veb. Janapharm Laborchemie, Apolda, Germany. Triolein, Stearic acid were obtained from Sigma Chemical Company, U.S.A.

Fat bodies from various life stages of *Chilo partellus* were isolated under ice cold Ringer solution. The fat bodies were cleaned with cold double distilled water, weighed and homogenized in cold double distilled water using a ground glass mortar and pestle. Homogenates were diluted with cold double distilled water so as to get 1% (wt/vol) concentration. Such homogenates were used for the assay of lipolytic activity. Lipase was assayed by the method of Hayase and Tappel (1970) except for free fatty acids.

The assay system contained 0.25 ml of substrate dispersed in gum acacia, 1.0 ml 0.1 M tris maleate buffer pH 8 for larval fat body and pH 7.6 for pupal fat body and 0.25 ml of suitable diluted 1% (wt/vol) enzyme solution in the total volume of 1.5 ml. Incubation was carried out in incubator shaker with continuous shaking for 30 minutes in glass stoppered conical flasks at 37 °C. At the end of incubation, the liberated fatty acids were measured colorimetrically according to Itaya (1977). The enzyme activity was expressed in μ-equiv. free fatty acids released/100 mg of fat body/30 minutes.

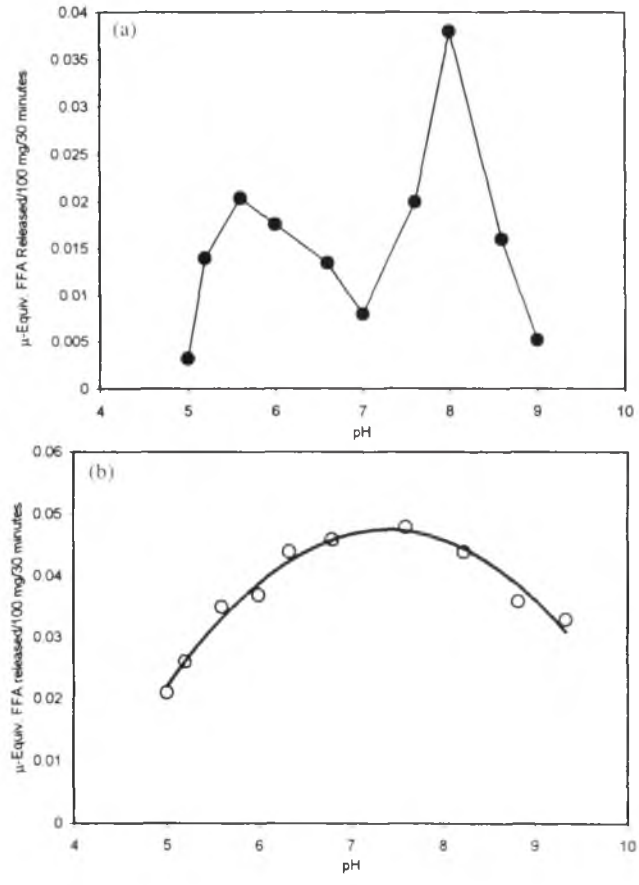

FIGURE 1. (a) Effect of pH on larval fat body lipase activity of *Chilo partellus* (Swinhoe), (b) Effect of pH on pupal fat body lipase activity of *Chilo partellus* (Swinhoe).

RESULTS

Larval developmental period of *Chilo partellus* is of 25-days and metamorphosis of 10-days. The larval fat body lipase showed pH optima at 5.6 and 8. The maximum lipase activity observed at pH 8 in 19-day larval and pH 7.6 in 4-day pupal fat body of *Chilo partellus* (Fig. 1).

Changes in lipase activity in fat body during larval growth and metamorphosis are shown in Fig. 2. The gradual increase in lipase activity in larval fat body was observed from 8 to 11-day, sharp fall from 11 to 12-day, gradual increase from 12-day to 20-day and decrease from 20 to 25-day. Maximum lipase activity was noted in 11-day larvae and minimum in 12 and 25-day larvae.

During metamorphosis, gradual increase in fat body lipase activity was observed from prepupa (Po) to 2-day pupa, sharp increase from 2 to 3-day pupa, decline in

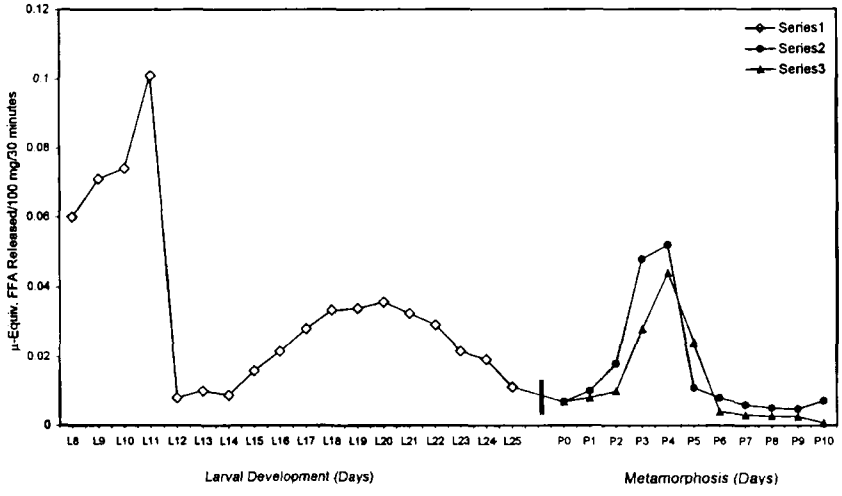

FIGURE 2. Lipase activity in fat body of *Chilo partellus* during larval growth and metamorphosis.

activity from 3-day pupa to 4-day. The sharp fall in enzyme activity from 4 to 5-day in male pupa and 4 to 6-day in female pupa,gradual decrease from 5 to 10-day in male pupa and 6 to 10-day in female pupa. Minimum activity noted in 10-day pupa and maximum in 4-day pupa.

DISCUSSION

Gilbert *et al.* (1965) noted the homogenate of male adult Cecropia fat body possesses a true lipase with a single pH optima at pH 7.2. The triglyceride lipase of fat bodies of Southern armyworm moth has a pH optimum of about 8 (Stevenson, 1972). In 7-day blowfly larvae, lipase activity in fat body was optimal over the pH range 7.5 to 8 (Price, 1975). The lipase activity in the fat body of *Chrysomyia rufifacies* during larval growth and metamorphosis was maximal at the broad pH range 8.5 to 9 (Pol and Sawant, 1997).

In the present work, larval fat body lipase showed pH optima at 5.6 and 8 suggesting the presence of acidic and alkaline lipase. The maximum fat body lipase activity was observed at pH 8 in 19-day larvae at pH 7.6 in 4-day pupae of *C. partellus*. This indicates that fat body lipase is maximally active at an alkaline pH. Our results agree with the findings of Gilbert *et al.* (1965); Stevenson (1972); Price (1975); Pol and Sawant (1997).

Most insects accumulate large quantities of lipid during larval period. The lipid is stored in the fat body in the form of triglycerides (Price, 1975). The lipid store is then utilized to provide energy for the metamorphosis (Rao and Agarwal, 1971).

The fat body contains triglycerol which may be released into the haemolymph for distribution to other tissues in the form of diacylglycerol by the action of

triacylglycerol lipase (Hoffman and Downer, 1979). Fatty acids are transported from the fat body to the site of utilization as diglycerides (Gilbert *et al.*, 1965). For utilization of lipids especially triglycerides for energy production the components of fatty acids must be hydrolized. Lipases are the enzymes which are responsible for such hydrolysis. The lipases present in the fat bodies and other body tissues are considered as extra-digestive lipases as they are present outside the alimentary canal (Nandanan *et al.*, 1973). Insect controls its fatty acid composition to meet the needs of individual tissues and ontogenetic constraints (Howard and Styanley-Samuelson, 1996). Dutkowski (1973) noted difference in the fat body lipolytic activity of two sexes.

Increase in the larval fat body lipase activity from 8 to 11-day suggest that during this early feeding period of larval development of *Chilo partellus*, the larvae are most active, the energy and structural components required for their active life and growth is supplied by triacylglycerol catabolism. Sharp fall from 11 to 12-day suggest the possibility of sudden inactive larval stage requiring minimum energy. Increase in larval fat body lipase activity from 12 to 20-day suggest the gradual feeding and developmental stage after third moult which requires the energy and the structural components. Decrease in enzyme activity from 20 to 25-day indicates the gradual inactivation of larvae entering into the pupal stage which require minimum energy. The maximum enzyme activity observed in fat body of 11-day larvae indicates the most active feeding larval stage requiring more energy and structural components for larval growth.

The gradual increase in fat body lipase activity from prepupa (Po) to 2-day pupa indicates the gradual increase of histolysis and sharp increase from 2 to 3-day pupa suggest the extensive histolysis and decline in activity from 3 to 4-day pupa suggest the decline in histolysis and just beginning of the histogenesis which provides energy for metamorphosis and materials for histogenesis. The maximum lipase activity observed in 4-day pupa suggest the maximum histolysis. The sharp fall in enzyme activity from 4 to 5-day in male and 4 to 6-day in female suggest the depletion of lipid. Slow decrease in activity from 5 to 10-day pupa in male and from 6-day pupa to 10-day pupa in female may be due to the depletion of lipids and complete histogenesis. The main source of energy and structural components during metamorphosis is lipid and the lipolytic activity is an instrumental in release of energy and structural components.

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REFERENCES

Chino, H. and Gilbert, L. I. (1964) Diglyceride release from insect fat body: a possible means of lipid transport, *Science* **143**: 359–361.

Conn, E. E. and Stumpf, P. K. (1969) Metabolism of biological compounds, In: *Outlines of Biochemistry*, Wiley Eastern Private Limited: New Delhi, 113–344.

Dutkowski, A. B. (1973) Sex differences in the lipolytic activity of Galleria mellonella fat body, *J. Insect Physiol.* **19**: 1721–1726.

Dixon, M. and Webb, E. G. (1979) Enzyme kinetics, In: *Enzymes*, Clay, R (Editor). The Chaucer Press Ltd.: Bungar, Suffolk, 47–206.

George, J. C. and Eapen, J. (1959b) Lipase activity in the fat body of the desert locust, *Schistocerca gregaria*, *J. Cell Comp. Physiol.* **54**: 293–295.

Gilbert, L. I., Chino, H. and Domroese, K. A. (1965) Lipolytic activity of insect tissue and its significance in lipid transport, *J. Insect Physiol.* **11**: 1057–1070.

Hayase, K. and Tappel, A. L. (1970) Specificity and other properties of lysosomal lipase of rat liver, *J. Biol. Chem.* **245**: 169–175.

Hoffman, A. G. D. and Downer, R. G. H. (1979) End product specificity of triacylglycerol lipases from intestine, fat body, muscle, haemolymph of American cockroach, *Periplaneta americana*. *Lipids.* **14**: 893–899.

Howard, R. W. and Styanley-Samuelson, D. W. (1996) Fatty acid composition of fat body and malpighian tubules of the tenebrionid beetle, *Zophobas atratus*: significance in Eicosanoid–Mediated Physiology, *Comp. Biochem. Physiol.* **115B**: 429–437.

Itaya, K. (1977) A more sensitive and stable colorimetric determination of free fatty acids in blood, *J. Lipid Res.* **18**: 663–665.

Nandanan, D. M., Sehgal, S. S. and Agarwal, H C (1973) Properties and distribution of extra digestive lipase in the beetle *Trogoderma*, *Insect Biochem.* **3**: 223–230.

Pol, J. J. and Sawant, V. A. (1997) Lipase activity in the fat body of *Chrysomyia rufifacies* during larval growth and metamorphosis, *Entomon* **22(2)**: 101–104.

Pol, J. J. and Sawant, V. A. (1999) Lipase activity in some tissues of blow fly, *Chrysomyia rufifacies* during larval growth and metamorphosis-II., *Adv. Biosci.* **18(1)**: 79–86.

Price, G. M. (1975) Lipase activity in third instar larvae of the blowfly, *Calliphora erythrocephala*, *J. Insect. Biochem.* **5**: 53–60.

Rao, K. D. P. and Agarwal, H. C. (1971) Lipids of Trogoderma III. Phospholipids and fatty acid composition during development with note on fatty acid biosynthesis, *Comp. Biochem. Physiol.* **39B**: 183–194.

Stevenson, E. (1969) Monoglyceride lipase in moth flight, *J. Insect Physiol.* **15**: 1537–1550.

Stevenson, E. (1972) Haemolymph lipids and fat body lipase of the Southern armyworm moth, *J. Insect Physiol.* **18**: 1751–1756.

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Comparative evaluation of chemical and botanical insecticides against termites

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ABSTRACT: Insecticides *viz.*, Imidacloprid 17.8 SL, chlorpyriphos 20 EC, lindane 20 EC, endosulfan 35 EC, cypermethrin 10 EC and phorate 10G and neem manure were tested against termites in pots. Imidacloprid 0.012% was effective upto 3 months but at 0.008 and 0.004% were effective upto 2 months only. Chlorpyriphos at 0.04% was effective upto 2 months but at 0.02 and 0.03% were effective upto one month only. Lindane at 0.03 and 0.04% and endosulfan at 0.08% were effective upto one month. All the above insecticides gave above 50% corrected mortality. Lindane 0.02%, endosulfan 0.07%, neem manure 50 g per pot, phorate 0.1 g a.i. per pot and cypermethrin 0.0025% were found least effective. Among botanical insecticides, Nimbicidine and Nemactin were effective upto two months while Rakshak, Multineem, Neemgourd and Vanguard were effective for short time upto one month. Field trial was conducted in mango orchards of Upeda, Ghaziabad and Rohenda, Bulandshahar, Uttar Pradesh, India. Imidacloprid 0.012%, chlorpyriphos 0.04% and lindane 0.04% were found most effective and gave 100% reduction in termite population upto five months. Imidacloprid 0.004%, chlorpyriphos 0.02%, lindane 0.02%, lindane 1.3% dust @ 100 g per tree and neem manure 500 g per tree were found less effective.

KEYWORDS: *Odontotermes obesus*, *O. assmuthi*, efficacy, persistence, residual toxicity, imidacloprid, neem based formulations

Mango *(Mangifera indica L)* is the most important fruit crop of India which constitute 65% (9.22 million t) of the world production of 15.7 million ton (Anonymous, 1992). Its productivity is 7.0 t/ha in Uttar Pradesh as compared to 8.31 t/ha of India (Yadav, 1997). Among the various insects infesting mango crop, termite is important soil pest.

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Termite feed on the main trunks in the soil galleries and this adversely affects the growth of the plant and fruit yield (Khanna *et al.*, 1956).

In the past, the only protection against termite was to irrigate the fields, preferably with water and crude oil emulsion which acts as an effective repellent for about two weeks. During 1940's persistent organochlorine (cyclodiene) insecticides used a barriers in soil, can provide protection throughout the growing season for annual crops or longer for perennial and tree crops and forestry. Prior to use of cyclodienes, highly toxic chemicals such as Paris green and Arsenates were used (Beeson, 1941; Harris, 1971). Increasing concern over damage to human health and the environment has now resulted in the banning of cyclodienes in many countries including India. Research to develop alternative chemical methods has centered on newer, less persistent insecticides. In the present study the efficacy and residual toxicity of insecticides has workout in field at different places as well as under laboratory condition.

MATERIALS AND METHODS

Efficacy and persistence of insecticides and neem based formulations against *Odontotermes obesus* Rambur

The experiment was conducted on November 8, 1998 to March 9, 1999 at Horticultural Research Centre (HRC), Patharchatta, Udham Singh Nagar. Six months old seedlings were grown in earthen pots (diameter 30 cm and height 26 cm) filled with 7 kg of soiled for this experiment. Desired concentrations of imidacloprid (Confidor 200 SL) chlorpyriphos (Dursban 20 EC), lindane (Kanodane 20 EC), endosulfan (Endocel 35 EC) and cypermethrin (Ripcord 10 EC) were prepared in one litre of water. These were applied by drenching in pots, while neem manure (50 g per pot) and phorate (0.1 g a.i. per pot) were mixed in soil. The nylon cage with iron wire frame was put on each earthen pot after releasing the termites. Efficacy and persistence of the insecticides was tested by releasing 30 termite workers and soldiers in 3:1 ratio after 4 hours and 1, 2, 3, 4 and 5 months of application of insecticides. Six neem based formulations, viz. Neemgourd, Nimbicidine, Multineem, Vanguard, Nemactin and Rakshak 1500 ppm were tested on May 13, 1999 at HRC, Patharchatta. Small pieces of fungus comb were treated with neem formulations and efficacy and persistence was tested by releasing 30 termite workers and soldiers in 3:1 ratio after 4 hours and 1, 2 and 3 months of application. Mortality of termites in both the experiments were recorded after 12 hours of releasing the termites. Each treatment was replicated three times in completely randomised design and corrected % mortality in both the experiment was calculated by following formula:

$$\text{Corrected \% mortality} = \frac{T - C}{100 - C} \times 100$$

where, T = the observed % mortality in treatment

C = % mortality in control.

Residual toxicity of insecticides against *Odontotermes assmuthi* Holmgren in mango orchard of Upeda (Ghaziabad)

Field trial was conducted during September 12, 1998 to February 16, 1999 in an orchard at Upeda (Ghaziabad). Mango orchard has Dashehari cultivar trees in the age group of 15 years in sandy loam soil. Three insecticides viz. imidacloprid (Confidor 200 SL) at concentrations of 0.004, 0.008 and 0.012%, chlorpyriphos (Dursban 20 EC) and lindane (Kanodane 20 EC) at concentrations of 0.02 and 0.04%. Insecticides were diluted in 10 litre of water used as soil drench around and on the tree trunk manually. Number of termites were counted in 500 g soil before treatment and later three, four and five months after application. Each treatment was replicated four times in randomized block design. Percent reduction in termite population over untreated check was calculated by following formula:

$$\text{Percent reduction} = \frac{C - T}{C} \times 100$$

where, C = population in untreated tree

T = population in treated tree.

Residual toxicity of insecticides against *O. obesus* Rambur in mango orchard of Rohenda (Bulandshahar).

The field experiment was conducted during September 14, 1998 to February 17, 1999 in an orchard at Rohenda (Bulandshahar) with trees of Dashehari cultivar in age group of 20 years in sandy loam soil. Five treatments i.e. imidacloprid at the concentration of 0.004, 0.008 and 0.012%, chlorpyriphos and lindane at 0.02 and 0.04%, lindane 1.3% dust at the rate of 100 g per tree and neem manure at the rate of 500 g per tree were tested. Imidacloprid, chlorpyriphos and lindane were diluted in 10 litre of water and were applied as drench. Lindane dust and neem manure (deoiled) were mixed in soil in one meter periphery of tree canopy. All these treatments were replicated four times including control with single tree/replication in a randomised block design. Percent reduction was calculated by the formula as given earlier.

RESULTS AND DISCUSSION

Efficacy and persistence of insecticides and neem based formations against *O. obsesus* Rambur

Efficacy and persistence of insecticides against *O. obesus* Rambur was tested on mango seedlings planted in earthen pots. After 4 hours of application, mortality of workers and soldiers was 100% in all insecticides (Table 1). After one month of application, highest corrected percent mortality were recorded on 0.012% imidaloprid (81.82) followed by 0.008% imidacloprid (73.88), 0.04% chlorpyriphos (72.95), 0.004% imidacloprid (65.26), 0.04% lindane (60.34), 0.03% chlorpyriphos (58.06) and 0.02% chlorpyriphos (52.54). The lower corrected percent mortality recorded with 0.0025% cypermethrin (48.51), phorate (47.91), neem manure (45.97), 0.07% endosulfan (44.13) and 0.02% lindane (43.47). After two months of application,

imidacloprid 0.012, 0.008 and 0.004% were most effective and have no significant difference. Other concentration of insecticides gave below 50 corrected % mortality except 0.04 percent chlorpyriphos (55.3). After three months of application, 0.012% imidacloprid was effective with 51.26 corrected % mortality and remaining insecticides has less than 50%. After four and five months of application, all the above insecticides gave below 50 corrected percent mortality and considered to be ineffective against termites. Storey (1995) reported that imidacloprid affected the social behaviour of the termite, reducing its feeding and resulting in eventual death. Akhtar and Sanwar (1993) reported that deltamethrin was most effective followed by chlorpyriphos, alpha cypermethrin and dieldrin against *Bifiditermes beesoni*. Atkinson (1989) reported that phorate at the rate of 0.1 g a.i. per tree was effective against termites. Mariconi *et al.* (1990) observed that endosulfan gave effective control against *Cornitermes cumulans*. Neem manure (50 g per pot), 0.07% endosulfan and 0.02% lindane gave poor performance.

Efficacy and persistence of neem based formulations were tested in plastic vials along with small pieces of fungus comb. On the basis of observation recorded after 4 hours of application, the highest corrected % mortality *viz.*, 90 was in treatment of Nimbicidine and Nemactin (Table 2). The second most effective neem formulation was Rakshak (66.97), which was at par with Multineem (62.87). Vanguard (51.51) was found least effective. After one month of application, Nimbicidine and Nemactin were found effective and remaining formulations gave below 50% corrected mortality. After two and three months of application, all formulations gave below 12 percent corrected mortality. Grace and Yates (1992) reported that commercial insecticide formulation of neem (Margoson-O) containing 0.03% azadirachtin and 14% oil at 100 ppm gave significant mortality for a short time.

Residual toxicity of insecticides against *O. assmuthi* Holmgren in mango orchard of Upeda (Ghaziabad)

After three months of application (Table 3) residual toxicity of imidacloprid 0.008 and 0.012%, chlorpyriphos and lindane 0.04% were found most effective and gave 100% reduction in termite population. Imidacloprid 0.004% gave 92.86% reduction in termite population followed by chlorpyriphos and lindane 0.02% with 89.95 and 87.73% reduction in termite population, respectively. After four months of application cent percent reduction in termite population was observed in 0.012% imidacloprid, 0.04% chlorpyriphos and 0.04% lindane. Imidacloprid 0.008% was giving 89.11% reduction in termite population followed by 0.004% imidacloprid (78.27), 0.02% chlorpyriphos (70.67) and 0.02% lindane (68.02). After five months of treatment, imidaclorpid 0.012%, chlorpyriphos and lindane 0.04% gave 100% reduction in termite population followed by 0.008% imidacloprid (75.45), chlorpyriphos 0.02% (69.05) and imidacloprid 0.004% (63.11). Lindane 0.02% was found significantly inferior to all other treatments.

TABLE 1. Efficacy and persistence of insecticides against *O. obesus* Rambur in pots

Treatments (%)	Mean corrected percent mortality after					
	Four hour	One month	Two months	Three months	Four months	Five months
Imidacloprid	100	81.91	70.80	49.43	12.22	0
0.004	(90.00)*	(65.26)	(57.51)	(44.67)	(19.99)	(0)
Imidacloprid	100	88.74	77.51	55.21	16.67	1.11
0.008	(90.00)	(73.88)	(61.98)	(48.08)	(23.84)	(3.50)
Imidacloprid	100	94.22	83.22	60.69	18.89	1.11
0.012	(90.00)	(81.82)	(66.09)	(51.26)	(25.53)	(3.50)
Chlorpyriphos	100	62.91	41.42	25.86	4.44	0
0.02	(90.00)	(52.54)	(39.92)	(30.35)	(11.99)	(0)
Chlorpyriphos	100	71.92	51.76	38.15	6.67	0
0.03	(90.00)	(58.06)	(46.01)	(38.05)	(14.64)	(0)
Chlorpyriphos	100	87.55	67.29	47.36	11.11	2.22
0.04	(90.00)	(72.95)	(55.30)	(43.47)	(19.17)	(4.99)
Lindane	100	47.36	20.31	3.33	0	0
0.02	(90.00)	(43.47)	(26.55)	(6.14)	(0)	(0)
Lindane	100	66.40	44.94	28.09	3.33	0
0.03	(90.00)	(54.69)	(42.07)	(31.84)	(8.49)	(0)
Lindane	100	75.25	56.25	40.46	8.89	0
0.04	(90.00)	(60.34)	(48.61)	(39.46)	(16.53)	(0)
Endosulfan	100	48.47	22.35	4.52	0	0
0.07	(90.00)	(44.13)	(27.69)	(10.07)	(0)	(0)
Endosulfan	100	68.55	49.42	33.49	5.56	1.11
0.08	(90.00)	(56.14)	(44.67)	(34.71)	(13.48)	(3.50)
Cypermethrin	100	56.02	35.98	21.19	2.22	0
0.0025	(90.00)	(48.51)	(36.85)	(26.35)	(4.99)	(0)
Neem manure	100	51.69	26.97	11.27	1.11	0
50 g per pot	(90.00)	(45.97)	(31.26)	(18.81)	(3.50)	(0)
Phorate 0.1 g	100	55.01	33.56	17.93	2.22	0
a.i. per pot	(90.00)	(47.91)	(35.16)	(24.48)	(4.99)	(0)
Control	0	0	0	0	0	
CD ($P = 0.05$)		13.76	9.71	12.64	8.94	5.85

* Data given in parenthesis are angular transform value.

Residual toxicity of insecticides against *O. obesus* Rambur in the mango orchard of Rohendra (Bulandshahar)

Cent–percent reduction in termite population was recorded in 0.012% imidacloprid, 0.04% chlorpyriphos and 0.04% lindane after 3, 4 and 5 months of application (Table 4). Imidacloprid 0.008% gave 100, 88.39 and 78.73% reduction in termite population after 3, 4 and 5 months of application, respectively. Imidacloprid 0.004% was giving 91.28% reduction followed by chlorpyriphos 0.02% (88.79), lindane 0.02% (86.99), lindane 1.3% dust (85.88) and neem manure (82.15) after 3 months

TABLE 2. Efficacy and persistence of neem based formulations against *O. obesus* Rambur

Treatments (1550 ppm)	Mean corrected % mortality after			
	4 hour	One month	Two months	Three months
Neemgourd	71.1	28.89	2.33	0
	(57.65)*	(31.58)	(4.99)	(0)
Nimbicidine	100	65.56	28.89	1.11
	(90.00)	(54.16)	(32.18)	(3.50)
Multineem	78.89	38.89	4.44	0
	(62.87)	(38.34)	(11.99)	(0.00)
Vanguard	61.11	27.78	1.11	0
	(51.51)	(30.77)	(3.50)	(0.00)
Nemactin	100	60.11	22.22	0
	(90.00)	(51.78)	(27.53)	(0.00)
Rakshak	84.44	48.89	14.44	1.11
	(66.97)	(44.35)	(21.75)	(3.50)
Control	0	0	0	0
CD ($P = 0.05$)	6.83	16.06	11.40	5.68

* Data given in parenthesis are angular transform value.

TABLE 3. Residual toxicity of insecticides against *O. assmuthi* Holmgren at Upeda.

Treatments (%)	Mean % reduction in termite population over control after		
	Three months	Four months	Five months
Imidacloprid 0.004	92.86	78.27	63.11
Imidacloprid 0.008	100	89.11	75.45
Imidacloprid 0.012	100	100	100
Chlorpyriphos 0.02	89.95	70.67	69.05
Chlorpyriphos 0.04	100	100	100
Lindane 0.02	87.73	68.02	54.8
Lindane 0.04	100	100	100
Control	0	0	0
CD ($P = 0.05$)	12.7	6.7	6.93

of application. After 5 months, imidaclorpid 0.004% (67.34) was found at par with chlorpyriphos 0.02% (61.75) and lindane 1.3% dust (60). Neem manure (50.68) was found least effective and statistically equal to lindane 0.02% (56.49) and lindane 1.3% dust (60).

Srivastava (1997) reported that 0.1% chlorpyriphos and 0.25% imidacloprid are useful to control the termite. In the present investigation, imidacloprid 0.012%, chlorpyriphos 0.04% and lindane 0.04% gave 100% reduction in termite population upto five months. Akhtar and Sanwar (1991) reported that chlorpyriphos at 100 and 200 ppm caused 97% mortality. Gul and Chaudhary (1989) stated that lindane and

TABLE 4. Residual toxicity of insecticides against *O. obseus* Rambur at Rohenda.

Treatments (%)	Mean % reduction in termite population over control after		
	Three months	Four months	Five months
Imidacloprid 0.004	91.28	79.04	67.34
Imidacloprid 0.008	100	88.39	78.73
Imidacloprid 0.012	100	100	100
Chlorpyriphos 0.02	88.79	74.07	61.75
Chlorpyriphos 0.04	100	100	100
Lindane 0.02	86.69	70	56.49
Lindane 0.04	100	100	100
Lindane 1.3% dust 100 g per tree	85.88	72.54	60
Neem manure 500 g per tree	82.15	64.36	50.68
Control	0	0	0
CD ($P = 0.05$)	16.03	11.17	9.77

chlorpyriphos at 0.25, 0.5 and 1% applied to soil around *Dalbergia sisso* trees and their bark gave complete protection upto 20 months. Thakur (1990) recommended that application of 0.2% chlorpyriphos and 10 g BHC 10% dust gave effective control in forest trees. The present studies showed that lindane 1.3% dust at the rate of 100 g per tree gave 85.88, 72.54 and 60% termite population reduction after 3, 4 and 5 months of application, respectively. Neem manure (500 g per tree) gave poor performance than the above insecticides and with 82.15, 64.36 and 50.68% termite population reduction after 3, 4 and 5 months of application, respectively.

REFERENCES

Akhtar, M. S. and Sanwar, G. (1993) Studies on toxicities of different insecticides against *Bifiditermes beesoni* Gardner, *Pakistan Journal of Zoology* **25(2)**: 93–8.

Akhtar, M. S. and Sanwar, G. (1991) Comparison of toxicity and repellency of dieldrin with other insecticides used against *Bifiditermes beesoni* Gardner (Isoptera), *Pakistan Journal of Zoology* **23(3)**: 269–71.

Anonymous, (1992) *FAO Production Year Book*, 179.

Atkinson, P. R. (1989) Controlled release insecticide granules, compared with other insecticides, for use against the termite, *Macrotermes natalensis* Haviland, in the establishment of Eucalyptus plantations, *Crop Protection* **8(6)**: 387–96.

Beeson, C. F. C. (1941) A guide to the control of termites for forest officers, *Indian Forest Records (New Series), Entomology* **4**: 44–90.

Grace, J. K. and Yates, J. R. (1992) Behavioural effect of a neem insecticides on *Coptotermes formosanus* (Iso:Rhinotermitidae), *Tropical Pest Management* **38(2)**: 176–80.

Gul, H. and Chaudhary, M. I. (1989) Efficacy of insecticides against termites infesting barks of living trees, *Proceedings of Pakistan Congress of Zoology* **9**: 61–3.

Harris, W. V. (1971) *Termites: Their Recognition and Control*(2nd edition), Longmans: London, 186.

Khanna, K. L., Nigam, L. N., Agarwal, S. B. D. and Butani, D. K. (1956) *Proc. International Society of Sugarcane Technologist 9th Congress, New Delhi*, 908–36.

Mariconi, F. A. M., Raizer, A. L., Arashiro, F. Y., Biondo, C. L. and Clari, A. I. (1990) Field test of insecticides against the mound termite, *Cornitermes cumulans* Kollar (Iso:Termitidae), *Annais de Sociedade Entomologica do Brasil* **19(1)**: 163–9.

Srivastava, R. P. (1997) *Mango cultivation*, International Book Distributing Co.,: Lucknow, India, 175–229.

Storey, G. (1995) Imidacloprid, a developing new termiticide chemisty, *Pest Control Magazine* 36–7.

Thakur, M. L. (1990) *Control of Termites in Plantation Foresty*, F. R. I. Press: Dehradun.

Yadav, I. S. (1997) Mango research in India ; the past 50 years, *Indian Horticulture* **42 (2)**: 10–17.

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Description of a new genus and two new species with record of a known species of Encyrtidae (Hymenoptera: Chalcidoidea), from the Andaman & Nicobar Islands, India

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ABSTRACT: A new genus of Encyrtidae, *Saucrencyrtus* gen. nov. (Type species, *S. insulanus* sp. nov.) and a new species of the genus *Paratetracnemoidea* Girault (*P insulana* sp. nov) are described. A key to the world species of *Paratetracnemoidea* is given. Also *Cheiloneuromyia javensis* Girault is recorded for the first time from India. The material for three species was collected from the Andaman & Nicobar Islands, India.

KEYWORDS: Hymenoptera, Encyrtidae from Andaman & Nicobar Islands (India), *Saucrencyrtus* gen. nov., *Paratetracnemoidea insulana* sp. nov., other encyrtids

INTRODUCTION

In the Indo-Pacific region the encyrtid fauna of India is second best known after Australia (Noyes and Hayat, 1984). About 160 genera of the family are recorded from India. But from Andaman & Nicobar Islands this insect group is very little known and represented by only 14 species of which Hayat and Singh (1999) recently recorded 8 species. Singh (1995) described a new genus *Manmohanencyrtus* from the islands. The species known from the islands are: *Adelencyrtus bimaculatus* Alam, *Aenasius advena* Compare, *Anagyrus mirzai* Agarwal & Alam, *A. subflaviceps* (Girault), *A. umairi* Noyes & Hayat, *Cheiloneurus insulus* Kaul & Agarwal, *C. yasumatsui* Trjapitzin, *Diversinervus cervantesi* (Girault), *Encyrtus aurantii* Geoffroy, *Leptomastix nigricincta* Risbec, *Manmohanencyrtus hayati* Singh, *Neocladia trifasciata* Singh & Agarwal, *Ooencyrtus lucens* Huang & Noyes, and *Prochiloneurus testaceus* (Agarwal).

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In the present paper we describe a new genus *Saucrencyrtus* with a new species, a new species of the Genus *Paratetracnemoidea* Girault, and record *Cheiloneuromyia javensis* Girault, all from the Andaman & Nicobar Islands.

***Saucrencyrtus* gen. nov.**

Type species: Saucrencyrtus insulanus sp. nov.

Female Head with frontovertex slightly convex, gradually rounded and merging with face, and slightly less than twice as wide as long; frontovertex very narrow, slightly less than one-sixth of head width; scrobes distinct, not deep, inverted 'U' shaped, with rounded sides and above; toruli less than their own lengths from mouth margin; malar sulcus present; malar space two-fifths of eye length; occipital margin above rounded; eyes just reaching occiput behind. Mandibles with two small teeth and a truncation (Fig. 3). Antenna (Fig. 1) 1, 1, 6, 3; with a distinct anellus; scape cylindrical, long; pedicel conspicuously longer, slightly more than 3x as long as broad, longer than F1; funicle segments all longer than broad; clava very slightly broader than F6, with rounded, untruncated apex.

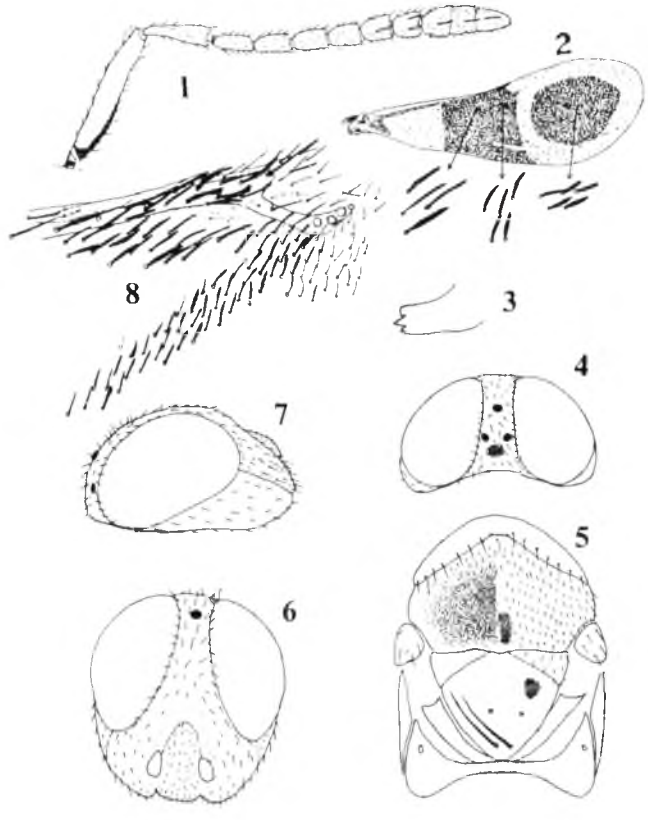
Thoracic dorsum slightly convex; pronotum transverse, posterior margin biconvex with a median rounded notch; mesoscutum with notaular lines absent; axillae distinctly separated being overlapped mesally by mesoscutum; scutellum nearly as long as mesoscutum and nearly as long as broad, with rounded apex; propodeum medially narrow, less than one-fifth scutellum length (Fig. 5); mesopleuron enlarged posteriorly and touching base of gaster, thus separating metapleuron from hind coxa.

Fore wing with infuscated areas with scale-like setae, hyaline areas largely with normal setae (Fig. 2); marginal vein not clearly separated from submarginal vein, but appears about as long as stigmal vein, the latter rather thin and with a slightly expanded stigma; postmarginal vein short (Fig. 8); costal cell narrow, with 6 setae distally, ventral surface with a line of setae and in proximal two-thirds a second line of short setae; basal triangle setose to base, without an asetose area; linea calva narrow, closed posteriorly by a line of setae. Legs rather long and slender, mid tibial spur a fifth shorter than basitarsus; tarsal formula, 5-5-5.

Gaster slightly shorter than thorax, sub-triangular, cercal plates situated in about basal two-sevenths; hypopygium extending to about three-quarters length along gaster, ovipositor not exserted.

Male Unknown

Comments The new genus does not run well to any genus in the keys to genera given by Trjapitzin (1989, Palaearctic genera) Noyes *et al.* (1997, Nearctic genera), but it runs to *Paraschedius* Mercet in the key to the Indo-Pacific genera by Noyes and Hayat (1984). But it definitely does not belong in this genus, or even to the tribe Habrolepidini in which *Paraschedius* is currently placed. We are unable to place this close to any of the known genera, except that it appears best placed in the tribe



FIGURES 1–8. *Saucrencyrtus insulanus* sp. nov., female: 1, antenna; 2, fore wing; 3, mandible; 4, head in dorsal view; 5, thorax in dorsal view; 6, head in frontal view; 7, head in profile; 8, part of fore wing enlarged to show venation and setation.

Microteryini, subtribe Microteryina. In the oriental region the tribe Microteryini is represented by 19 genera.

Etymology Greek, *Saukros* = beautiful, graceful + Encyrtus.

***Saucrencyrtus insulanus* sp. nov. (Figs 1–8)**

Female

Length, 1.20 mm. Body varicolored; head yellow with very faint brown suffusions on frontovertex, temple and malar space; vertex with golden bronzy shine; pronotum concealed part brown, collar golden yellow to yellow brown; mesoscutum, except narrowly the sides which are whitish and yellowish posterior margin, golden bronzy in anterior one-third and bluish-green in posterior two-thirds; axillae golden; scutellum yellow golden with a slight brownish tinge in distal two-thirds; metanotum and

propodeum brownish yellow; prepectus white; mesopleuron white with about distal third light brown; gaster dark brown, faintly bronzy violet, base narrowly, sides of T-I, T-II and sterna, except last sternum, pale yellow to white, Palpi white. Tips of mandibles reddish. Antenna; radicle pale brown with apex dark: scape, pedicel and flagellum white, ventral margin in basal half of scape dark brown. Fore wing with infuscation as in Fig. 2; the infuscation behind venation pale brown, whereas the more or less oval infuscated patch on disc dark, smoky; hind wing hyaline. Legs, including coxae, white, with yellow to pale yellow brown fore and mid tibiae; fore tarsi and mid knee pale brown; hind femur brownish in distal third.

Frontovertex with moderately deep, fine (small) polygonally reticulate sculpture; mesoscutum finely reticulate, sculpture deeper than on scutellum; scutellum with sculpture similar to that on frontovertex; rest of body appears smooth, but with very fine reticulate sculpture visible at higher magnification. Setae on frontovertex short, pale brown, those along anterior third of each eye margin and on malar space, white; pronotal collar with longer, dark setae; mesoscutum rather densely setose, setae white; scutellum with setae appear detached, but a pair at apex very long and dark brown, length of seta nearly 0.7x of scutellum length (6 : 8.5).

The above description and the following relative measurements are sufficient to recognize this species.

Relative measurements: *(From card)*

Head dorsal width (length), 32 (18); frontovertex width at narrowest, 5; head in profile, length, 20; height, 32; eye length, 25; malar space length, 10; torulus length, 5; torulus to mouth margin distance, 4. Distance between posterior ocelli, 2. 25; distance from a posterior ocellus to occipital margin, 5; distance from a posterior ocellus to anterior ocellus, 4. Thorax length, 42; mesoscutum length (width), 18 (27); scutellum length (width), 17 (18); propodeum median length, 3; distance between propodeal spiracles, ca. 22. Gaster length (width), 36 (29); cercal plates from base, 10; from base of gaster to apex of hypopygium, 28. (From slide): Scape length, 31; pedicel length, 14; F4-6, 20; clava, 19. Wing length (width), 125 (44). Mid tibia length, 62; mid basitarsus, 23; mid spur, 17.5

Male Unknown.

Host Unknown.

Distribution India: Andaman & Nicobar Islands.

Holotype ♀, (on card, with right antenna and wings, and left legs on a slide); INDIA: Andaman & Nicobar Islands, Sipighat, 10. iv. 1991, collected by Sudhir Singh. Deposited in National Insect Reference Collection at Forest Entomology Division, Research Institute, Dehra Dun. Accession No. 20969.

Genus *Paratetracnemoidea* Girault

Paratetracnemoidea Girault, 1915: 166. Type species: *Paratetracnemoidea breviventris* Girault, by monotypy and original designation.

Rhinoencyrtus Mercet, 1918: 234. Type species; *Rhinoencyrtus malenotti* Mercet, by monotypy. Synonymy by Noyes & Hayat, 1984: 318.

This well-known genus, earlier known by its junior synonym, *Rhinoencyrtus* Mercet (see Noyes and Hayat, 1984), is characterized by the presence of a metallic body with a strongly convex thorax, presence of a spoon-shaped 'tooth' on face above mouth margin between toruli; apically pointed mandible (with one long tooth and a short, receding tooth); submarginal vein of fore wing distally bifurcated into an anterior, short vein (marginal!) and a long stigmal vein; stigmal sensilla arranged in a square; gaster clearly shorter than thorax; absence of paratergites; and hypopygium not reaching to apex of gaster. Slide-mounted external genitalia of the female show that the third valvulae are fused with second valvifers (Fig. 9)

The genus in known from an unnamed species from the Indian mainland (Noyes and Hayat, 1984).

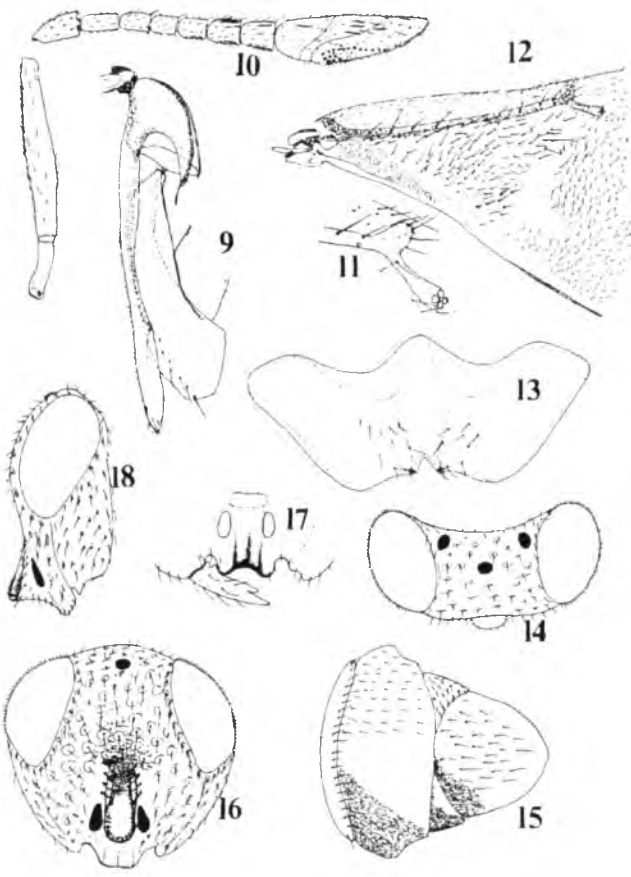
***Paratetracnemoidea insulana* sp. nov. (Fig. 9–18)**

Female

Length, head and thorax, 1.22 mm. Body dark, metallic, frontovertex intense bluish-green with violet on vertex; facial 'tooth', seen from above (spoon-shaped) bluish-green with coppery margin; frontovertex and face with large, circular setigerous punctures; pronotum in posterior half finely reticulate, with the cells mostly transversely drawn-out; mesoscutum with raised reticulations, which become slightly longitudinally drawn-out in distal fourth, bronzy-violet and faintly bluish-green; scutellum with longitudinal reticulations, dull black, with margins polished and smooth; mesopleuron light bronzy; gaster dark brown, with bluish-green to violet shine. Tips of mandibles dark reddish brown. Antennal radicle dark brown, with violet shine; scape yellow-brown with brownish infuscation along dorsal margin; pedicel and flagellum dark brown. Wings hyaline; fore wing with faint infuscation at base, and with almost indistinct infuscation on disc beyond venation. Legs dark brown except narrowly apex of fore and hind tibiae and apical third of mid tibiae which are testaceous yellow; tarsal segments 1–4 of fore and mid legs yellow, those of hind legs yellow brown to brown; last tarsal segments of all legs dark brown.

Setae on frontovertex and face, white; on malar space behind sulcus, pale brown; on mesoscutum and sides of propodeum white; and on axillae and scutellum (apical pair long) golden brown.

The above description, illustrations and the following relative measurements are sufficient to recognize this species.



FIGURES 9–18. *Paratetracnemoidea insulana* sp. nov., female: 9, ovipositor; 10, antenna; 11, venation of fore wing; 12, basal part of fore wing showing venation and setation; 13, hypopygium; 14, head in dorsal view; 15, thorax in dorsal view; 16, head in frontal view; 17, mounth margin with mandible; 18, head in profile.

Relative measurements *(From card)*

Head dorsal width, 43.5; frontovertex width, 19; scape length 23; head frontal length (width), 42 (43); eye length (width), 24 (16); malar space length, 14; head profile length, 18; height, 42. Thorax dorsal length, 42; pronotum length (width), 3 (36); mesoscutum length, (width), 18 (40); scutellum length (width), 22 (24); distance between propodeal spiracles, 34. Mid tibia length, 40. (From slide): Fore wing length (width), 141 (61); venation, 61. T-VII length, 45; ovipositor length, 74.5. Mid tibia length, 65; mid basitarsus length, 20; mid spur length, 20.

Male Unknown.

Host Unknown.

Distribution India: Andaman & Nicobar Islands.

Holotype ♀, (on card, with one antenna, wings of one side, legs dissected gaster, on one slide: INDIA: Andaman & Nicobar Islands, Car Nicobar, Mallaika, 3. iv. 1991, Collected by Sudhir Singh, deposited in National Insect Reference Collection at Forest Entomology Division, Forest Research Institute, Dehra Dun. Accession No. 20970.

Comments The genus contains 4 described species: *P. breviventris* Girault, from Australia; *malenotti* (Mercet) from the Palaearctic region; *cornis* Prinsloo, from South Africa; and *americana* Gordh, from the USA. All these species, including the new species described here, are rather very closely related, differing mainly in the relative dimensions of the flagellar segments; head dimensions; body sculpture, especially the presence or absence of large, thimble-like punctures on the head; and the intensity of the fore wing infuscation or its near absence. (See Mercet, 1918, 1921; Gordh, 1985; Prinsloo, 1986; Dahms and Gordh, 1997 for Girault's species.)

The new species, *insulana*, is most closely related to *cornis* and *malenotti*, from which it differs by the characters given in the key.

Key to species of *Paratetracnemoidea*, females

1. Frontovertex and face with deep sculpture, but without large, setigerous pits . . 2
   Frontovertex and face with large, setigerous pits 3
2. Body black, with faint metallic purple reflections; mesoscutum and gaster appearing coppery in some plays of light; scape yellow brown; legs except black coxae, yellow brown . . . . . *breviventris* Girault
   Body black, with weak blue-coppery reflections; antenna including scape dark reddish brown; coxae and femora black, tibiae somewhat more pale . *americana* Gordh
3. Fore wing with pale infuscation from base to apex with two darker areas, one behind apex of venation and the other rather broader subapical band; scape black, and at least slightly more than 7x as long as broad 4
   Fore wing with pale, almost indistinct infuscation from base to apex; scape yellow brown, with brownish infuscation along dorsal margin, and about 6.5x as long as broad. (Head about 2.3x as broad as frontovertex; clava as long as F2-6 combined) . . . . . *insulana* sp. nov.
4. Frontovertex with a large median circular area devoid of pits; clava as long as F2-6 combined . . . . . *cornis* Prinsloo.
   Frontovertex uniformly pitted, without a large median circular area devoid of pits; clava as long as F3-6 combined . . . . . *malenotti* (Mercet)

***Cheiloneuromyia javensis* Girault**

Cheiloneuromyia javensis Girault, 1916: 480–481, female. Indonesia, Java, Salatiga (U. S. National Museum, Washington, D. C.). Noyes and Hayat 1984: 248, taxonomy.

Specimens examined India: Andaman & Nicobar Islands, Mallaika, Car Nicobar, 2 ♀, (one on slide), 3. iv. 1991, coll. Sudhir Singh.

Comments The genus *Cheiloneuromyia* was known from India from undetermined material (Noyes and Hayat, 1984). This is the first record of *C. javensis* from India.

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REFERENCES

Dahms, E. and Gordh, G. (1997) A review of the genera of Australian Encyrtidae (Hymenoptera: Chalcidoidea) described from Australia by A. A. Girault with a checklist of included species, *Mem. Entomol. International* **9**: 518 p.

Girault, A. A. (1915) Australian Hymenoptera Chalcidoidea-VII, *Mem. Queensland Mus.* **4**: 1–184.

Girault, A. A. (1916) New Javanese chalcidoid Hymenoptera, *Proc. United States Natl. Mus.* **51**: 479–485.

Gordh, G. (1985) A new species of *Paratetracnemoidea* Girault, 1915, found in North America, with a discussion of generic placement (Hymenoptera: Encyrtidae), *Fla. Ent.* **68**: 587–594.

Hayat, M. and Singh, S. (1999) New records of some Encyrtidae (Hymenoptera: Chalcidoidea) from the Andaman & Nicobar Islands, India, *Bionotes* **1(3)**: 56.

Mercet, R. G. (1918) Generos nuevos de Encirtinos de Espana, *Boln. R. Soc. Esp. Hist. Nal.* **18**: 234–241.

Mercet, R. G. (1921) Fauna Iberica. Himenopteros Fam. Encirtidos, *Mus. Nac. Cienc. Natl. Madrid* 727 p.

Noyes, J. S. and Hayat, M. (1984) A review of the genera of Indo-Pacific Encyrtidae (Hymenoptera: Chalcidoidea), *Bull. British Mus. Natl. (Ent.)* **48**: 131–395.

Noyes, J. S., Woolley, J. B. and Zolnerowich, G. (1997) Encyrtidae, In: *Annotated Keys to the Genera of Nearctic Chalcidoidea (Hymenoptera)*, Gibson, G. A. P., Huber, J. T. and Woolley, J. B. (Editors). NRC Research Press: Ottawa, chapter 8.

Prinsloo, G. L. (1986) On some poorly known Southern African Encyrtidae (Hymenoptera: Chalcidoidea), with descriptions of four new species, *J. Ent. Soc. Southern Africa* **49**: 121–136.

Singh, S. (1995) *Manmonahencyrtus*, a new encytrid genus from Andaman Islands, India and, notes on the genus *Chrysoplatycerus* Ashmead (Hymenoptera: Chalcidoidea: Encyrtidae), *Oriental Insects* **29**: 161–173.

Trjapitzin, V. A. (1989) Parasitic Hymenoptera of the Fam. Encyrtidae of Palaearctics, *Opred. Faune. SSSR, No. 158* (in Russian).

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Spatio-temporal changes in the infestation of citrus leafminer, *Phyllocnistis citrella* Stantion in Meghalaya

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ABSTRACT: The spatial and temporal changes of the leafminer, *Phyllocnistis citrella* Stantion infestation on citrus was studied on the different flushes of citrus at ICAR Research Complex for NEH Region farm, Barapani. Various indices of dispersion like variance mean ratio, David and Moore index, exponent k of the negative binomial, mean crowding, patchiness index were used to study the distribution pattern of *P. citrella*. Patchiness regression index was calculated and Taylor's power law was used to know the type of distribution of the leafminer infestation. The *P. citrella* infestation followed Negative binomial (contagious) distribution with low to medium damage during the first two flushes and Poisson distribution at higher infestation levels during the third flush. Correlation coefficients were calculated and significant differences in infestation patterns with weather were observed in the three flushes. Different Multiple Linear Regression equations were found fit to explain the leafminer *P. citrella* infestation in different flushes of citrus in Meghalaya.

KEYWORDS: Citrus leafminer, *Phyllocnistis citrella*, Spatial distribution, dispersion, weather effects

INTRODUCTION

The North Eastern Hill (NEH) region is unique in its geography and origin compared to rest of India and like-wise the diversity of insect species. Khasi mandarin (*Citrus reticulata* Blanco) is grown extensively in this region and is attacked by innumerable number of insect species, which are contributing to citrus decline. Citrus leafminer, *Phyllocnistis citrella* Stantion is one among such factors contributing to decline of citrus and occurring in severe epidemics every year (Pathak *et al.*, 1999). Its incidence was recorded upto 70–100% (Anonymous, 1982) and its damage ranges from 10–40% on different varieties (Azad Thakur and Gupta, 1991). Its damage is increasing

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because of more number of flushes in this region when compared to other parts of citrus growing regions of India. Understanding the distribution pattern of *P. citrella* is a prerequisite for its successful management.

The distribution of insect species or dispersion of its population or disposition of them in space and time is of considerable ecological significance. The distribution of such organisms is used to describe the condition of the population with respect to its habitat. Consideration of dispersion pattern along with changes in size is vital in interpreting population dynamics. Such studies will be helpful in understanding predator-prey and host-parasite relationships (Anderson, 1974; Crofton, 1971; Murdie and Hassell, 1973; Hassell and May, 1974). For example, if a mortality factor reduces the clumping of a sessile organism this is an indication that it acts most severely on the highest densities or if the dispersion of a population becomes more regular then intensification of a competition should be suspected (Iwao, 1970). Complex biological processes and various environmental factors determine the size of insect population at a given time. Hence, studies on the distribution or dispersion changes in the infestation levels of *P. citrella* were conducted on the three flushes of citrus in Meghalaya from 1999 to 2000.

MATERIALS AND METHODS

Three terminal twigs were selected from each citrus tree and the number of leaves infested by leafminer was counted. Citrus in this region is having three flushes. The first flush appears in January–February, second in June–July and the third in September and October. The first two flushes are the major ones and the 3rd flush is minor. The weather during the first flush period is dry-cooler, second flush rainy and the third is humid–winter. The twigs having new flushes were selected for the study. Each twig is approximately having 30–40 leaves. Observations on the number of leaves damaged by citrus leafminer in each new twig was recorded. Three twigs were selected on each tree and 11 trees were selected for recording infestation levels. Observations were started at monthly intervals from February to June for the first flush; at fortnightly intervals for the remaining two flushes (July–August and September–December). Only new twigs were selected during all the three flushes and the old twigs were discarded for the infestation counts.

Various indices of dispersion were used to analyse the *P. citrella* infestation without any *a priori* assumption of the type of distribution. Three basic units used for fitting the distribution were mean ($\overline{X}$), variance (s^2) and the number of samples (n or N) on which the mean is based. The mean infestation ($\overline{X}$) and the variance was calculated for each new flush. The simplest approach used was variance mean ratio (VMR). The value of VMR is one for Poisson distribution and less than one for regular or positive binomial and more than one for aggregated or negative binomial distribution. The index of clumping of David and Moore (1954) was calculated with $I_{DM} = s^2/\overline{X} - 1$, which gives a value of zero for a random, positive value for negative binomial and negative for positive binomial. The value of 'k' of the negative binomial which is the measure of the amount of clumping and is often referred to as the dispersion

parameter was calculated with the method of Katti and Gurland (1962). As the values of k becomes larger, the distribution approaches Poisson, while fractional values of k indicate a distribution tending towards the logarithmic series, which occurs when $k = 0$. As the observations were recorded during different seasons of a year, and to ease the comparison of these, common k was calculated by moment or regression method (Bliss, 1958). A further graphical test of the homogeneity of the samples was done by plotting $1/k$ against the $\overline{X}$ for each new flush.

The relationship between s^2 and $\overline{X}$ of a series of samples were done with Taylor's power law $S^2 = a\overline{X}^b$ (Taylor *et al.*, 1978). The concept of mean crowding is used to indicate the possible effect of mutual interference or competition among individuals, which is expected, when they encounter one another. The sample estimate of mean crowding (X^*) was obtained by $X^* = \overline{X} + (s^2/\overline{X} - 1)$. The ratio of mean crowding to mean density ($X^*/\overline{X}$) is called patchiness (Lloyd, 1967) whose value is less than one, equal to or larger than one in under-dispersed, random and clumped distribution, respectively. The Iwao's patchiness regression-index $X^* = \alpha + \beta\overline{X}$ was also calculated over a range of densities (Iwao, 1972). Mean size of infestation per twig (C^*) was calculated (Tanigoshi *et al.*, 1975). The constant α which is the intercept on X^* axis is called the index of basic contagion. The coefficient β is related to the pattern in which the pest utilizes its habitat and is called density contagiousness coefficient. The distribution with $\alpha = 0$ and $\beta > 1$ corresponds to the negative binomial series with a common k. The distribution with $\alpha = 0$ and $\beta = 1$ corresponds to models of randomly distributed colonies. The coefficient of variation (CV) was also calculated.

The relationship between infestation pattern (Y) and weather parameters *viz.*, maximum temperature (X_1), minimum temperature (X_2), relative humidity (X_3), rainfall (X_4) and sunshine hours (X_5) was calculated and multiple regression analysis (MLR) of this data was carried out for each flush.

RESULTS AND DISCUSSION

Various dispersion indices were calculated based on mean and the variance of the infestation levels for each new flush and were presented year wise i.e. 1999 (Table 1) and 2000 (Table 2). During the initiation of each flush the number of leaves damaged by the *P. citrella* was less and gradually increased to the end of the season. Very high values of mean infestation was recorded during second and third flush and the damage continued upto November. During both the years and in the first two flushes the variance (s^2) was greater than the mean and further the VMR indicated that the distribution of the damage pattern was contagious or clumped. But in the third flush the VMR was almost unity showing randomness even though the proportion of the damage was similar to that of first two flushes. Most commonly in ecological studies, the s^2 is found to be larger than the mean i.e. the distribution is contagious or over-dispersed and were expressed by the negative binomial (or Pascal) distribution (Harcourt, 1965).

The k of the negative binomial was also low (around 3 to 5) during both the years and in the first two flushes. But in the 3rd flush of 1999 and from 2nd flush of 2000,

TABLE 1. Dispersion indices of citrus leafminer *Phyllocnistis citrella* Stantion infestation during the three flushes of 1999

Month	Mean ($\overline{X}$)	Variance	VMR	k	$1/k$	I_{DM}	X^*	$X^*/\overline{X}$	C^*	CV
I Flush										
Feb.	04.85	11.82	2.438	3.371	0.296	1.438	06.28	1.296	07.28	0.709
Mar.	07.42	22.85	3.077	3.572	0.279	2.077	09.50	1.279	10.50	0.644
Apr.	08.96	27.24	3.040	4.391	0.227	2.040	11.00	1.227	12.00	0.582
May	10.96	34.63	3.159	5.074	0.197	2.159	13.12	1.464	14.12	0.536
June	11.82	39.84	3.371	4.984	0.200	2.371	14.19	1.200	15.19	0.534
				$K_C = 4.493$						
II Flush										
July I	07.36	23.50	3.192	3.360	0.297	2.192	09.55	1.297	10.55	0.658
July II	09.21	25.52	3.097	4.388	0.227	2.097	11.30	1.227	12.30	0.580
Aug. I	11.72	38.19	3.257	5.188	0.193	2.257	13.98	1.192	14.98	0.527
Aug. II	12.87	46.28	3.594	4.953	0.202	2.594	15.47	1.201	16.47	0.528
				$K_C = 4.592$						
III Flush										
Sept. I	03.30	02.03	0.642	008.56	0.116	0.385	02.91	0.883	03.91	0.431
Sept. II	07.12	07.56	1.061	115.22	0.008	0.061	07.18	1.008	08.18	0.386
Oct. I	08.64	08.89	1.030	284.65	0.003	0.030	08.66	1.003	09.66	0.345
Oct II	10.69	09.91	0.926	145.01	0.006	0.074	10.62	0.993	11.62	0.294
Nov. I	12.72	15.26	1.200	071.58	0.014	0.200	12.91	1.015	13.92	0.307
Nov. II	14.06	17.08	1.214	065.45	0.015	0.215	13.98	0.995	14.98	0.294
				$K_C = 72.96$						

the index of aggregation (k) of the infested leaves was very higher indicated that the infestation of citrus leafminer at lower densities followed contagious distribution and at higher densities it followed Poisson distribution. This was well proved with other dispersion parameters. The index of clumping showed positive values (>1) during the first two flushes indicated contagious nature of *P. citrella* damage and with values <1 during third flush indicated randomness of the infestation. Many contagious insect populations are well explained by negative binomial series (Ibarra *et al.*, 1965).

The smaller the values of k the greater the extent of aggregation, whereas a large value (over about 8) indicated that the distribution is approaching a Poisson i.e. virtually random (Southwood, 1978). In the present study the value of k was not constant in all the months and often increased with the mean. *P. citrella* damage followed negative binomial series at low densities and Poisson at higher densities. This was well supported by the variance is almost equal to the mean in the third flush during both the years. Although, at low mean density many insects follow a distribution that doesn't differ from Poisson, it doesn't necessarily mean that insects are distributed randomly under such conditions. Generally distribution of any insect during initial phase of infestation is random and becomes contagious during later phases. The phenomenon with the *P. citrella* was found quite opposite. Similarly, with the Nantucket pine tip moth (*Rhyacionia frustrana*), as the population density

TABLE 2. Dispersion indices of citrus leaf miner *Phyllocnistis citrella* Stantion infestation during the three flushes of 2000

Month	Mean ($\overline{X}$)	Variance	VMR	k	$1/k$	I_{DM}	X^*	$X^*/\overline{X}$	C^*	CV
I Flush										
Feb.	4.606	09.93	2.156	3.980	0.251	1.156	05.76	1.251	06.76	0.683
Mar.	6.636	14.72	2.217	5.450	0.183	1.217	07.85	1.183	08.85	0.578
Apr.	8.545	24.73	2.796	4.388	0.227	1.796	10.34	1.210	11.34	0.582
May	8.757	25.39	2.899	4.608	0.217	1.899	10.65	1.216	11.65	0.575
June	9.939	27.75	2.792	5.546	0.180	1.792	11.73	1.180	12.73	0.530
				$K_c = 4.849$						
II Flush										
July I	11.48	28.61	2.491	07.69	0.130	1.491	12.97	1.129	13.97	0.465
July II	14.00	33.57	2.398	10.01	0.099	1.398	15.39	1.099	16.39	0.413
Aug. I	17.45	38.91	2.229	14.18	0.070	1.229	18.67	1.070	19.67	0.357
Aug. II	19.30	39.18	2.029	18.75	0.053	1.029	20.33	1.053	21.33	0.324
				$K_c = 12.820$						
III Flush										
Sept. I	03.03	03.13	1.033	089.12	0.011	0.034	03.06	1.011	04.06	0.584
Sept. II	05.06	05.12	1.011	084.33	0.011	0.011	05.07	1.002	06.07	0.447
Oct. I	06.03	06.45	1.069	087.29	0.014	0.069	06.09	1.011	07.09	0.421
Oct. II	07.63	08.32	1.089	084.85	0.011	0.089	07.71	1.010	08.71	0.377
Nov. I	11.96	13.10	1.114	103.33	0.009	0.114	11.87	1.009	12.87	0.308
Nov. II	15.14	16.40	1.083	181.92	0.005	0.083	15.22	1.005	16.22	0.267
				$K_c = 128.19$						

increases still further the distribution tends towards Poisson again, i.e. the k values becomes high (Waters, 1959). The population of several forest insects shows a tendency to become more random at higher densities.

The patchiness index values during both the flushes was >1.0 confirmed the negative binomial series of the infestation and was equal to one in third flush indicated a random distribution. Mean number of infested leaves per twig (C^*) also increased with the mean.

The CV values were approaching unity during first two flushes and approaching zero during the second/third flush signifies the contagious distribution during first two flushes and random in the third flush. It was also observed that the CV values were higher during the beginning of infestation and became lessened with the increase in infestation. Clearly the smaller the k, the larger was the CV. The degree of aggregation, which k expresses could well affects the influence of predators and parasites. The aggregation recognized by the negative binomial may be due to either active aggregation by the insect or to some heterogeneity of the environment. In the present study, as all the values of k were greater than 2 indicating either of the factor governing this phenomenon.

A graphical test was done to test the homogeneity of the samples between the flushes by plotting $1/k$ against the $\overline{X}$ for each flush. Neither a trend nor clustering

was found and the fitting of common k was justified. Very high values of K_c during the third flush indicated the distribution of the infestation of *P. citrella* was random as the age of the flush hardens. It was hypothesized that, by the time of occurrence of second and third flush, the pest migrate from old flush to the newly emerging leaves and contribute to additive damage and when a branch is taken into account, almost all the leaves will be damaged by the time of appearance of third flush and the pest distributes randomly to the leaves because of competitiveness.

Iwao's patchiness regression ($X^* = \alpha + \beta\overline{X}$) also confirmed the dual nature of the *P. citrella* infestation. During both the flushes the value of index of basic contagion (α) was >0 and the value of density contagiousness coefficient (β) was >1 indicated the contagious nature of infestation of leafminer (Table 3). But during the third flush of 1999 and 2000 the value of β was approximately equal to zero or less than zero (negative) confirmed the shift of the distribution of the infestation from negative binomial to the Poisson at higher densities.

The Taylor's power law ($S^2 = a\overline{X}^b$) also showed the same pattern. The parameter 'b' of the power law is a measure of aggregation that is generally considered characteristic and constant for the species (Taylor *et al.*, 1978). High values of $b(>1)$ during the three flushes of 1999 and 1st flush of 2000 show strong contagion, but crossed the Poisson line ($b = 1$) showed a change to random distribution at higher density. Various indices of the distribution thus confirm the fit of the negative binomial series for the leafminer infestation during first two flushes and the Poisson for the last flush. Various studies indicated that insects follow negative binomial series (Shukla, 1986; Shukla and Pathak, 1987). High values of coefficient of determination (r^2) explained a perfect relationship between the mean and the variance values and a good fit for the two regression equations with leafminer infestation.

Relationship between leafminer infestation and the weather parameters

As the value of the exponent k of the negative binomial was more than two, it was opined that weather factors might also have contributed to differential pattern of leafminer infestation apart from the active biological process of the pest on citrus, because the region is characterized by lower temperatures, high humidity and very high rainfall throughout the year except in the months of December to February. These weather changes could have well contributed to these changes.

The relationship between the different weather parameters and the density of leafminer infestation was correlated and presented in Table 4. During both the years, a characteristic relationship between infestation and maximum temperature, minimum temperature and rainfall was observed during the three flushes. The correlation coefficient (r) was positive in the first flush crossed zero value in second flush and became negative in the third flush. It indicated that the weather factors, which were conducive for the leafminer damage during February–June, were not conducive from September to November. It is clear that with the increase in maximum temperature, minimum temperature, relative humidity and rainfall the damage increased in the first flush and conversely with the decrease in the above parameters the pest damage had

TABLE 3. Regression parameters of the spatial distribution of *Phyllocnistis citrella* Stantion

Regression	1999			2000		
	I Flush	II Flush	III Flush	I Flush	II Flush	III Flush
Taylor's Power law $S^2 = a\overline{X}^b$						
Sampling parameter (a)	0.1761	0.3288	−0.4097	0.0351	0.7948	−0.0117
Aggregation parameter (b)	1.3259	1.1867	1.4326	1.4305	0.6310	1.0465
Coefficient of determination (R^2)	0.9930**	0.9778**	0.9810**	0.9768**	0.9662**	0.9986**
Correlation coefficient (r)	0.9964**	0.9888*	0.9904**	0.9883**	0.9828*	0.9992**
Iwao's Patchiness Regression $X^* = \alpha + \beta\overline{X}$						
Index of basic contagion (α)	1.0039	1.5986	−0.3352	0.3912	2.1691	−0.3700
Density contagiousness coefficient (β)	1.1144	1.0667	1.0312	1.1534	0.9433	1.0758
Coefficient of determination (R^2)	0.9981**	0.9973**	0.9986**	0.9961**	0.9881**	0.9966**
Correlation coefficient (r)	0.9990**	0.9986**	0.9992**	0.9980**	0.9999**	0.9982**

*Significant at 5% level. **Significant at 1% level.

TABLE 4. Relationship between infestation of leafminer *Phyllocnistis citrella* Stantion with weather parameters

Year	Flush	*df*	Max. Temp. X_1	Min. Temp. X_2	R.H. X_3	Rainfall X_4	Sunshine hours X_5
1999	I	3	0.2636	0.9558**	0.6149	0.841*	−0.9595**
	II	2	0.0711	−0.1700	0.0110	−0.593	0.2800
	III	4	−0.8906*	−0.9220*	−0.0370	−0.580	−0.9750**
2000	I	3	0.9571*	0.9965**	0.0436	0.8692	−0.8496
	II	2	−0.7770	−0.7734	0.8880	0.7933	−0.8604
	III	4	−0.6128	−0.9772*	0.0260	−0.7961	0.9076*

*Significant at 5% level. **Significant at 1% level.

TABLE 5. Multiple regression (MLR) analysis

Year	Flush	Equation	Coefficient of Multiple determination (R^2)
1999	I	$Y = 12.63+0.3654X_1 - X_2 - 0.1098X_3 + 0.1313X_4 - 1.0722X_5$	1.0
	II	$Y = -128.59 - X_1 - 3.8163X_2 + 2.1741X_3 - X_4 + 7.8234X_5$	1.0
	III	$Y = -25.59 - 1.2570X_1 + 0.6231X_2 + 0.5428X_3 - 0.3213X_4 + 1.8908X_5$	1.0
2000	I	$Y = 9.5398 + 2.907X_1 + 0.3052X_2 - 0.0217X_3 - 0.0169X_4 - 0.6667X_5$	1.0
	II	$Y = -1315.82 + 15.339X_1 + 47.335X_2 + 1.8215X_3 + 1.5325X_4 - 23.186X_5$	1.0
	III	$Y = 41.90 - 0.3848X_1 - 0.8821X_2 - 0.1419X_3 + 0.0157X_4 + 0.4650X_5$	1.0

increased. Such phenomenon was rarely observed. The same pattern was observed with sunshine hours. The damage was more in 3rd flush with lowering minimum temperatures ($r = -0.9772^*$) and the relatively high sunshine hours (around 5–7) during this period might have been conducive for the increase in damage. But during first flush, with the decrease in sunshine hours because of very high rainfall, the pest damage increased as evidenced by very high negative relationship during the years 1999 and 2000 ($r = -0.9595^{**}$ and -0.8496).

Multiple regression analysis showed very high value of multiple coefficient of determination (R^2) during the three flushes. The best fitted multiple regression equations (Table 5) which were different during the three flushes and all the weather parameters together contributing to the differential distribution pattern of the leafminer infestation.

It was concluded from the results that the citrus leafminer, *P. citrella* infestation varies in different flushes with negative binomial distribution at low densities and

Poisson distribution at higher densities. The MLR with weather parameters have also proved the above pattern with differential correlation coefficients. These studies are useful in assessing the intensity of infestation during different flushes and for designing the management practices. Such studies are more helpful in understanding the bioecology of citrus leafminer bioagents and their release protocols for its management at higher altitudes.

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REFERENCES

Anderson, R. M. (1974) Population dynamics of the cestode *Caryophyllaeus laticeps* (Palls, 1781) in the ebream (*Abramis brama* L.), *J. Anim. Ecol.* **43**: 305–321.

Anonymous (1982) Mandarin orange decline in NEH Region and its control *Technical Bulletin No. 16*, ICAR Research Complex: Shillong, India, 32 p.

Azad Thakur, N. S. and Gupta, S. G. (1991) Screening of citrus germplasm against leafminer *(Phyllocnistis citrella)* and its control in mid hills of Meghalaya, *Ind. J. Appl. Ent.* **5**: 41–46.

Bliss, C. I. (1958) The analysis of insect counts as negative binomial distribution, *Proc. X Int. Cong. Ent.* **2**: 1015–1032.

Crofton, H. D. (1971) A quantitative approach to parasitism, *Parasitology* **62**: 179–193.

David, F. N. and Moore, P. G. (1954) Notes on contagious distributions in plant population, *Ann. Bot. Lond. N.S.*, **18**: 47–53.

Harcourt, D. G. (1965) Spatial pattern of the cabbage looper, *Trichplusia ni* on crucifers, *Ann. Ent. Soc. Am.* **58**: 89–94.

Hassell, M. P. and May, R. M. (1974) Aggregation of predators and insect parasites and its effect on stability, *J. Anim. Ecol* **43**: 567–594.

Ibarra, E. L. O., Wallwork, J. A. and Rodriguez, J. G. (1965) Ecological studies of mites found in sheep and cattle pastures. I. Distribution patterns of orbatid mites, *Ann. Ent. Soc. Am.* **58**: 153–159.

Iwao, S. (1970) Analysis of contagiousness in the action of mortality factors on the western tent caterpillar population by using the m*-m relationship, *Res. Popul. Ecol.* **12**: 100–110.

Iwao, S. (1972) Application of the m*-m method to the analysis of spatial patterns by changing the quadrant size, *Res. Popul. Ecol.* **14**: 97–128.

Katti, S. K. and Gurland, J. (1962) Efficiency of certain methods of estimation for the negative binomial and the Neyman type A distribution, *Biometrika* **49**: 215–226.

Lloyd, M. (1967) Mean crowding, *J. Anim. Ecol.* **36**: 1–30.

Murdie, G. and Hassell, M. P. (1973) Food distribution, searching success and predator-prey models, In: *The mathematical Theory of the Dynamics of Biological Populations*, Barlett, M. S. and Hiorns, R. W. (Editors). Academic Press: London, 87–101.

Pathak, K. A., Rajasekhara Rao, K., Mishra, M., Gupta, S. G. and Yadav, D. S. (1999) Integrated management of citrus leaf miner, *Phyllocnistis citrella* Stantion in Meghalaya, In: *Hi-Tech Citrus Management (Proc. Int. Sym. Citriculture (Nov. 23–27, 1999)*, Singh, S. and Ghosh, S. P. (Editors). NRC for Citrus, Nagpur: India, 889–894.

Shukla, R. P. (1986) Spatial pattern of Egg plant shoot and fruit borer, *Leucinodes orbonalis* Guen. (Lepidoptera: Pyraustidae) larvae, *Insect Sci. Appl.* **7(5)**: 611–615.

Shukla, R. P. and Pathak, K. A. (1987) Spatial distribution of corn leaf aphid, *Rhopalosiphum maidis* (Fitch) and its predator *Coccinella septumpunctata* Linn., *Indian J. Agric. Sci.* **57(7)**: 487–489.

Southwood, T. R. E. (1978) *Ecological Methods*, The ELBS and Chapman & Hall: London. 524 p.

Tanigoshi, L. K., Browne, R. W. and Hoyt, S. C. (1975) A study on the dispersion pattern and foliage injury by *Tetranychus medinieli* (Acarina: Tetranychidae) in simple apple ecosystem, *Can. Ent.* **107**: 439–446.

Taylor, L. R., Woiwod, I. P. and Perry, J. N. (1978) The density dependence of spatial behaviour and the variety of randomness, *J. Anim. Ecol.* **47**: 383–406.

Waters, W. E. (1959) A quantitative measure aggregations in insects, *J. Econ. Ent.* **52**: 1180–1184.

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Studies on the effects of Diethylstilbestrol, a synthetic non-steroidal Estrogen on silkworm *Bombyx mori* L.

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ABSTRACT: The improvement of commercial characteristics and the seed quality in silkworm rearing are the twin aspects of research and developmental activities in sericulture. As a continuum of this effort an attempt is made to study the effect of Diethylstilbestrol (DES) a synthetic non-steroidal estrogen on larval growth, commercial characteristics and fecundity in silkworm, *Bombyx mori*. Fifth instar larva of *Bombyx mori* (LXB_4 D_2) were sexed into male and female and subjected to topical application of 0.01, 0.025 and 0.050 μg/larva of DES. The larval growth was found to increase in all the groups treated with DES. Significant changes in commercial characteristics of the cocoon were observed both in male and female. A dosage of 0.025 μg/larva had induced a significant increase in shell weight in males in contrast to an increase in the pupal weight in females. The enhancement of protein synthesis in the treated larva is reflected in the haemolymph protein profile (10% SDS-PAGE). DES has significantly increased the weight of the ovary as well as number of eggs in the ovary. The results obtained in the investigation clearly indicate that DES is effective in influencing both the cocoon characteristics and fecundity in silkworm, *Bombyx mori*.

KEYWORDS: Diethylstilbestrol, *Bombyx mori*, commercial characteristics, storage proteins

INTRODUCTION

The most important physiological process in silkworm growth and silk production is nutrition. Provision of nutritionally rich mulberry leaves to the silkworms plays a significant role in the development and cocoon production (Fukuda, 1959). Various nutrients like proteins, amino acids, minerals, vitamins, sugars, antibiotics etc.

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have been supplemented along with mulberry leaves to improve silk production (Kumararaja *et al.*, 1972; House, 1974; Horie and Watanabe, 1983; Madhavan *et al.*, 1984; Krishnan *et al.*, 1995). The metabolic significance of vertebrates steroids (testosterone, estradiol-17β and progesterone) has been identified in the haemolymph of silkworm *Bombyx mori* (Venkata Rami Reddy *et al.*, 1994). Recently, Keshan and Ray (2000) reported the possible role of estradiol-17 & β on the enhancement of silk production in *B. mori*. De Loof and De Clerk (1986); and Swevers *et al.* (1991) proposed that more attention should be given on the physiological relevance of classical vertebrate sex hormones in insects. These present paper attempts to study the effect of Diethylstilbestrol (DES), a non-steroidal estrogen analogue (Emmens, 1964) on the haemolymph protein, the development, the commercial characteristics, and fecundity of silkworm *B. mori*.

Any change occurring in the body of the test animal will be reflected in the haemolymph protein. In the larvae of *B. mori* the storage proteins are the major haemolymph proteins that are synthesized by the larval fat body and play an important role as reservoirs of amino acids utilized during pupal and adult development (Tojo *et al.*, 1980; Levenbook, 1985). These storage proteins are highly sensitive to nutrition, hormones, temperature etc (Janarthanan *et al.*, 1999). Thus the study of storage protein with respect to DES in *B. mori* would serves as an important marker for investigating the development and commercial characteristics.

Experimental animal

Commercial seeds (disease free layings) of silkworm *Bombyx mori* ($LXNB_4D_2$) were obtained from Government Grainage, Tiruchirappalli, Tamil Nadu, India. The eggs were, incubated and larvae were reared by adopting paraffin paper rearing method and shelf rearing method (Krishnaswami *et al.*, 1970) MR-2 mulberry variety was used to feed the worms. Freshly moulted V instar larvae weighing between 0.7 to 0.95 g were separated into 2 groups as male and female for further studies.

Determination of effective dosage of Diethylstilbestrol

Diethylstilbestrol in the form of tablet (Sigma Chemicals Company, St. Louis, U.S.A.) was used in this study. 10 mg DES was dissolved in 1 ml of ethanol and kept as stock. The required dosages were prepared from the above stock solution. In a preliminary study different dosages of DES namely 100, 10, 1, 0.1 and 0.01 μg/larva were topically applied on the mid-dorsal region of the worm from 0 day of V instar onwards. Since higher mortality, decrease in body weight and growth retardation were observed in the larvae at higher dosages (>0.1 μg) and not at 0.1 μg the dosages were reduced to 0.01 and 0.05 μg/larva to assess the impact.

Freshly moulted V instar larvae were divided into 5 groups. Each group consisted of 3 replicates of 15 worms each. One group served as control and the other group treated with ethanol served as positive control i.e. placebo (The vehicle of the hormone). The remaining 3 groups were topically applied with 0.01, 0.025 and 0.05 μg/larva of DES. The dosages were given every day at 10 a.m. from 0 day of V instar onwards. The

topical application of acetone (placebo) did not induce significant change in any of the parameters studied, the results pertaining to the effect of placebo are not presented in the results and discussion.

Larval growth and commercial characteristics

The larval weight was determined every day till the worms started to spin. The cocoon weight, pupal weight and shell weight were determined individually in a top loading monopan balance with an accuracy of 0.1 mg.

Total haemolymph protein and its profile

The haemolymph samples were collected from the control and treated groups every day at 10 a.m. from 1-day onwards. 150 μl of haemolymph of was collected in an eppendorf tube prerinsed with phenylthiourea by making a cut in the first prolegs and equal a mount of tris buffer of pH 7.2 was added and centrifuged for 3 minutes at 3000 rpm to remove the haemocytes and other cell debris. The collected samples were stored at $-20\,^{\circ}C$ in a deep freezer till further analysis. The total haemolymph protein content was estimated by adopting Bradford (1976) method.

Electrophoretic separation: 10% SDS-PAGE analysis

The haemolymph protein was subjected to 10% SDS-PAGE under denaturing conditions (Laemmli, 1970). Simultaneously marker protein (Bio-Rad, USA) were run for comparison. The gels were stained using Coomassie brilliant blue dye (0.25 g Coomassie Blue R-250, 25% methanol, 7% acetic acid) for 5 hours and destained in destaining solution (Methonal 30%, Acetic acid 10% and H_2O 60%) to visualize the polypeptides. Then the gels were subsequently stored in 7% acetic acid in dark.

Determination of ovary weight (g) and number of eggs in the ovary

The female moths (15 numbers) were dissected out and ovary weight of each moth was determined in a monopan balance after removing the haemolymph in a blotting paper. The number of eggs in each ovary was counted with the help of hand lens.

Statistical analysis

The results obtained were tested for significance using '*t*' test (Zar, 1999).

RESULTS

Effect on larval growth

Table 1 illustrates the growth profile of the V instar larvae of *B. mori*. Control male and female larvae grew upto a maximum of 3.04 and 3.59 g respectively on 6-day, stopped feeding and began to spin on 7-day. Whereas both male and female larvae treated with DES reached a maximum weight of 3.58 and 3.84 g (0.025 μg) and 3.6 and 3.7 g (0.05 μg) on 5-day, stopped feeding and started to spin on 6-day. Irrespective of sex, the treated larvae were found to be heavier than control ($P < 0.05$) and started spinning on 20–24 hrs earlier than that of control.

TABLE 1. Effect of topical application of DES on body weight (g) of V instar larvae of *B. mori*

Treatment		Age of V instar (day)															
		0		1		2		3		4		5		6		7	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Control		0.728 ±0.17	0.948 ±0.10	0.959 ±0.10	1.480 ±0.11	1.560 ±0.08	2.160 ±0.10	1.980 ±0.09	2.60 ±0.09	2.270 ±0.15	3.38 ±0.05	2.900 ±0.11	3.49 ±0.05	3.040 ±0.017	3.59 ±0.12	2.800 ±0.05	3.31 ±0.1
Dosages of DES μg/larva	0.01	0.705 ±0.10	0.930 ±0.09	0.989 ±0.06	1.580 ±0.1	1.71 ±0.06	2.19 ±0.08	2.26 ±0.12	2.79 ±0.08	2.50 ±0.9	3.42 ±0.09	3.10 ±0.12	3.51 ±0.109	3.170 ±0.01	3.6 ±0.17	3.010 ±0.09	3.42 ±0.13
																$^*P < 0.05$	$^*P > 0.05$
	0.025	0.700 ±0.05	0.910 ±0.09	0.870 ±0.06	1.63 ±0.011	1.780 ±0.06	2.21 ±0.9	2.510 ±0.12	2.81 ±0.08	2.900 ±0.10	3.64 ±0.16	3.580 ±0.09	3.84 ±0.19	3.250 ±0.06	3.70 ±0.25	—	—
														$^*P < 0.05$	$^*P < 0.05$		
	0.050	0.732 ±0.05	0.910 ±0.09	1.028 ±0.06	1.54 ±0.12	1.810 ±0.09	2.14 ±0.15	2.42 ±0.10	2.72 ±0.16	2.910 ±0.210	3.41 ±0.18	3.600 ±0.10	3.72 ±0.2	3.300 ±0.170	3.60 ±0.26	—	—
														$^*P < 0.05$	$^*P < 0.05$		

The values are mean ± SD of 15 larvae for each test group. *Difference in the mean between control and DES treatment prior to spinning. $P < 0.05t'$ - value significant, $P > 0.05t'$ - value insignificant, M - Male, F - Female.

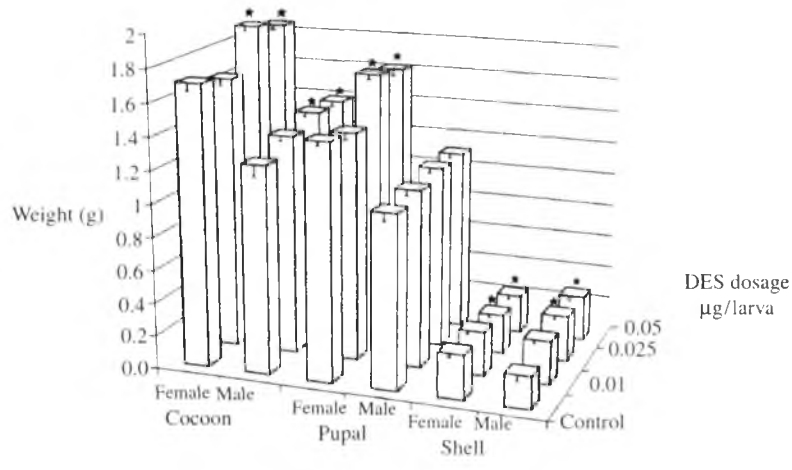

FIGURE 1. Changes in cocoon weight (g), pupal weight (g) and shell weight (g) in *Bombyx mori* subjected to topical application of DES during V instar. The values are mean ± SD of a sample size of 3 replicates of 15 worms each $*t'$–values significant. ($P < 0.05$).

Effect on commercial characteristics

Figure 1 shows the effect of DES on cocoon characteristics. Both the male and the female larvae subjected to topical application of DES produced cocoons of greater weight than that of control. The females generally produced heavier cocoons (1.71 ± 0.11 g) than that of males (1.26 ± 0.24 g). The females treated with 0.025 and 0.05 μg had produced significantly larger pupae than the control. However, the increase in the pupal weight in DES treated male was not significant.

Significant variations in shell weight were noted in male and female. The treated males had shown a significant increase in shell weight at all dosages, however the females shell weight had declined significantly at the dosages of 0.025 and 0.05 μg (Fig. 1). The shell ratio had also increased from 15.59% in control to a maximum of 19.78% in males at a dosage of 0.025 μg, whereas in female the shell ratio had decreased from 15.67% noted in control to 12.3% in the larvae treated with 0.05 μg DES (Fig. 2). 0.025 μg of DES is reported to have a maximum influence on the growth and commercial characters when compared to other 2 dosages namely 0.01 and 0.05 μg.

Effect on haemolymph protein content in female larvae

Results on the influence of DES on total haemolymph protein of V instar female larvae are presented in Fig. 3. The total haemolymph protein had increased significantly on 3, 4 and 5 days of V instar in the DES treated female. The changes in the haemolymph protein content of the control and treated larvae during V instar have shown a more or less similar trend i.e. drastic increase from 1-day, reaching a peak a day prior to spinning and decline on the day of spinning (Fig. 3).

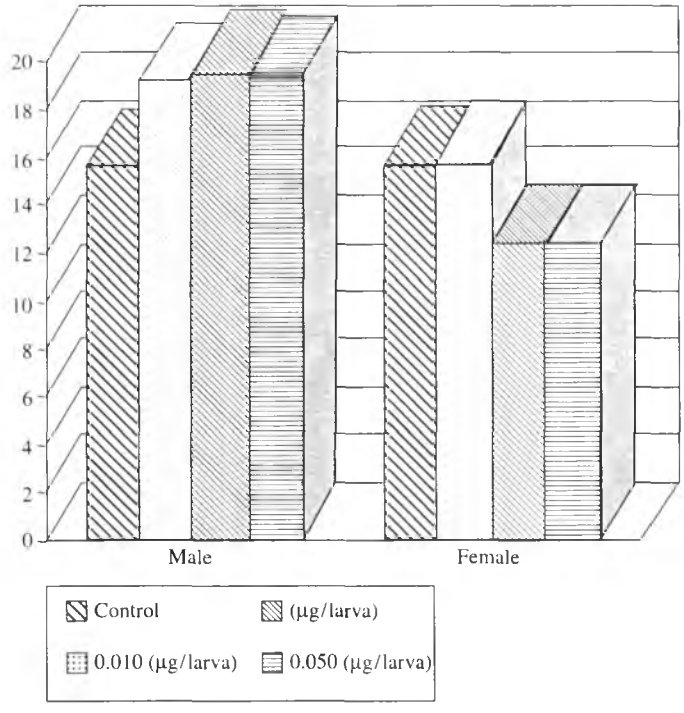

FIGURE 2. Changes in shell ratio (%) of male and female *Bombyx mori* subjected to topical application of DES during V instar. The values are based on a sample size of 3 replicates of 15 worms each.

Changes in protein profile

The protein profile of the haemolymph (10% SDS-PAGE) showed several polypeptides with molecular weight ranging from 200 kDa–20 kDa both in the control and in the DES treated groups. In all sample, 7 polypeptide constituted the bulk of the Coomassie brilliant blue stained fractions with the apparent molecular weights of 200, 82, 76, 72, 31, 29, and 26 kDa. A polypeptide localised around 200 kDa region was identified as heavy subunits of lipophorin. The three polypeptides resolved at 80 kDa region were identified as storage proteins that is storage protein 1 (SP 1) (82 kDa) and storage protein 2 (SP 2) (76 and 72 kDa). The 3rd group of polypeptide localised at 30 kDa region were found to be the 2nd most abundant haemolymph protein.

The appearance of 30 kDa fraction in the treated larva on 3-day (lane T_3 of Fig. 4) when compared to control on 4-day (lane C_3 Fig. 4) could be considered as an evidence for the induction of protein synthesis by DES. The pattern of storage protein accumulation 7-day of control (lane C_7 of Fig. 4) was similar to the pattern of storage protein accumulation on 6-day of DES treated larvae (lane T_6 of Fig. 4).

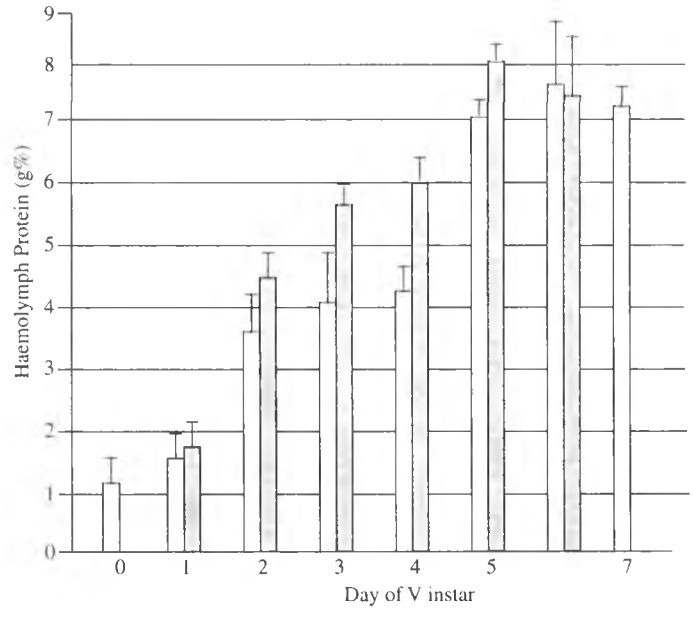

FIGURE 3. Influence of DES (0.025 μg) on haemolymph protein content (g%) of female V instar larvae of *Bombyx mori*. The values are mean ± SD of a sample size 10. $*t'$ - value significant ($P < 0.05$). □ Control, ■ DES (0.025 μg/larva).

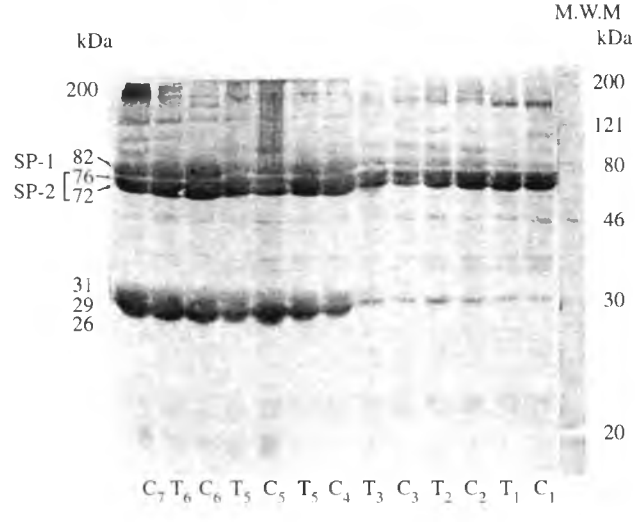

FIGURE 4. 10% SDS-PAGE of haemolymph protein profile (Stained with Coomassie blue) of female V instar larvae of *Bombyx mori*. C_1–C_7 Control larvae on 1 to 7-days. T_1–T_6 larvae treated with DES from 1 to 6-days. SP1 - storage protein 1 and SP2 - storage protein 2. MWM - molecular weight marker, kDa - kilo Dalton.

TABLE 2. Effect of topical application of DES on ovary weight (g) and no. of eggs in the ovary in *B. mori*

Parameter	Control	Dosages of DES		
		0.01 μg	0.025 μg	0.05 μg
Ovary weight (g)	0.403 ± 0.07	0.440 ± 0.06 $P > 0.05$	0.580 ± 0.08 $P < 0.05$	0.520 ± 0.05 $P < 0.05$
Number of eggs/female	422.8 ± 55.25	427.25 ± 28.8 $P > 0.05$	620.41 ± 76.8 $P < 0.05$	582.5 ± 77.2 $P < 0.05$

The values are mean ± SD of 15 female moth for each test group. $P > 0.05t'$ - value insignificant, $P < 0.05t'$ - value significant.

Effect on fecundity

Influence of DES on the weight of the ovary and the number of eggs are presented in Table 2. DES treatment had increased the ovary weight significantly in all the treated groups where the increase was maximum at 0.025 μg level. But there was a slight decline in the ovary weight among the larval groups treated with 0.05 μg. In accordance with this the number of eggs in the ovary also showed a similar trend.

DISCUSSION

Our continued effort to search for the effectiveness of DES in influencing the growth, commercial characteristics and fecundity, were prompted by the previous reports of Venkata Rami Reddy *et al.* (1994) who have reported the metabolic significance of vertebrate type steroids in silkworm *B. mori*. In the present investigation it was found that topical application of DES could influence the above mentioned parameters significantly. The DES treatment had not only enhanced the growth by increasing the body weight (g) but also shortened the feeding period. Investigation of Bradbury (1970) indicated that estradiol induces a moderate increase in the amount of dry mass/tissue unit. Significant increase in body weight supports the fact that DES brings about the same anabolic effect in *B. mori* as in the case of vertebrates, where growth promotion in DES administered animals were reported by Cowey *et al.* (1973) and Nirmala and Pandian (1983).

The increase in the larval weights had been reflected in the changes in the cocoon weight, pupal weight and shell weight. More reserves accumulated during feeding period allowed the worms to spin larger cocoons with greater weight when compared to control. These results could be supported from the reports Keshan and Ray (2000) who have shown an enhancement of cocoon characters in silkworm *B. mori* topically treated with estradiol-17 & β. The most salient finding of this study is the significant increase in the pupal weight and decrease in the shell weight of the female treated with 0.025 and 0.05 μg/larva of DES. It is suggested from these findings that the greater reserves accumulated during the feeding period is utilized towards the formation of pupal tissue than towards the formation of shell in the female. On the contrary, the accumulated reserves during feeding period is assumed to be utilized more towards

the formation of shell in the male. The consistent increase in the pupal weight and decrease in the weight and shell ratio in female prompted us to study the effect of DES on total haemolympth protein and its profile in the female.

The results make it clear that there is an increase in the levels of storage proteins SP 1 and SP 2 from the first day till the last day of V instar treated with DES. The results are in line with the findings of Tojo *et al.* (1980, 1981, 1985) and Mine *et al.* (1983). In several species of insects, the synthesis of haemolymph storage proteins during larval stage is attributed to the influence of hormones (Nijhout, 1975; Tojo *et al.*, 1981, 1985; Cymboroski *et al.*, 1982; Bosquet, 1983; Ismail and Dutta-Gupta, 1988). Janarthanan *et al.* (1999) have also reported that injection of methoprene repressed the accumulation of storage protein while 20-hydroxyecdysone have increased the same.

Stilbestrol being an estrogen analoge, it must be responsible for the increase in the total haemolymph protein content. Changes in the haemolymph protein profile during mid-instar further render support to the enhanced protein synthesis in the treated larvae. 30 kDa protein which is resolved in the control larvae on 4-day and their increase on subsequent days are in good agreement with the findings of Izumi *et al.* (1981); Sakai *et al.* (1988) and Vanishree *et al.* (1999). Whereas the same protein fraction made its appearance on 3-day in the treated larva presumed to be an evidence for the enhanced synthesis of protein a day in advance in the DES treated larva.

Increased synthesis of 30 kDa in the *B. mori* were reported by Janarthanan (1995) in larvae fed on the tender leaves of Ichinose variety. Lower concentration of this protein is reported in the larva during starvation (Janarthanan *et al.*, 1999). However, there has been little documented information about the impact of hormones on 30 kDa proteins in silkworm larvae. The 30 kDa proteins are believed to be actively involved in the formation of yolk granules in developing oocyte (Zhu *et al.*, 1986; Sakai *et al.*, 1988; Chen and Yamashita, 1990; Fujiwara and Yamashita, 1990). The results of the haemolymph protein profile of 7-day which is similar to the protein profile of the treated larva on 6-day. Conclusively prove that DES treatment had induce protein synthesis at an earlier stage and allowed the larvae to accumulate more reserves and spin cocoons in advance when compared to control.

From the results of this study, it appears that DES at 0.025 μg/larvae had induced the best increase in the ovary weight and fecundity. But application of higher dosage of 0.05 μg/larvae had let to a decrease in ovary weight and fecundity. These results are in accordance with the results obtained on the effect of DES on growth and food conversions in common carp *Cyprinus carpio* (Nanjundappa and Varghese, 1989). Further they have also discussed that, high level of DES appears to act catabolically resulting in growth depression. In addition, the results on increase in pupal weight and an increase in fecundity obtained here confirms the results of Rahman *et al.* (1978); Mukherjee *et al.* (1983); Gowda *et al.* (1988) and Kotikal *et al.* (1989) who have reported that adult of *B. mori* emerging from heavy females lay more number of eggs than light females. Siddique *et al.* (1985); Singh and Prasad (1987) and Nagalakshmamma *et al.* (1988) have reported a positive correlation between

pupal weight and egg production in eri silkworm *Samia Cynthia ricini*, *Anthraea mylitta* and *Philosomia ricini* respectively. Jayswal *et al.* (1991) had also observed that an increase in pupal weight increased the number of eggs laid in female insects. Similarly, highly significant correlation between female weight and fecundity has been observed in a bivoltine race of *B. mori* (Shaheen *et al.*, 1992). At present the studies related to the effect of DES on individual egg weights, hatchability and resistance of larva to infection are under consideration in our laboratory. The results of the present investigation strongly suggest that DES a synthetic non-steroidal estrogen had considerably influenced and enhanced the growth in silkworm. However, selection of optimum dosage of DES in practical sericulture needs careful monitoring.

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REFERENCES

Bosquet, G. (1983) Nutritional and non-nutritional stimulation of protein synthesis in the fat body of *Bombyx mori*, *Insect Biochem.* **13**: 281–288.

Bradbury, T. J. (1970) Direct action of estrogen on the ovary of the immature rat, *Endocrinol.* **68**: 115–120.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein—dye binding, *Anal. Biochem.* **72**: 248–254.

Chen, Y. I. and Yamashita, O. (1990) Non selective uptake of different 30 kDa plasma proteins by developing ovaries of the silkworm, *Bombyx mori*, *J. Seric. Sci., Jpn.* **59**: 202–209.

Cowey, C. B., Pope, A. J., Adron, J. W. and Blair, A. (1973) Studies on the nutrition of marine flatfish. The effect of oral administration of diethylstilbestrol and cyproheptadine on the growth of *Pleuronectes platessa*, *Mar. Biol.* **19**: 1–6.

Cymboroski, B., Mieczysalama, B., Beckage, N. E., Williams, C. M. and Riddiford, L. (1982) Juvenile hormone titres and metabolism during starvation induced supernumerary larval moulting of the tobacco hornworm, *Manduca sexta*, *J. Insect Physiol.* **28**: 129–135.

De Loof, A. and De Clerk, D. (1986) In: *Advances in Invertebrate Reproduction*, Prochet, M., Andries, J. C. and Dhainout, A. (Editors). Elsevier Science Publishers: B.V., Amsterdam, 117–123.

Emmens, C. W. (1964) In: *Methods in Hormone Research*, Dorfman, R. I. (Editor). Academic Press: New York, **3**: 14–27.

Fujiwara, Y. and Yamashita, O. (1990) Purification, characterization and development changes in the titer of a new larval serum protein of the silkworm, *Bombyx mori*. *J. Insect Biochem.* **21**: 751–759.

Fukuda, T. (1959) The correlation between the mulberry leaves taken by the silkworm, the silk protein in the silk gland and silk filament, *Bull-sericult. Exp. Sta.* **15**: 595–610.

Gowda, B. L., Sannaveerappanavar, V. J. and Shiva Yogeshwar, B. (1988) Fecundity and hatchability in mulberry in silkworm *Bombyx mori* L. as influenced by pupal weight, *International Congress on Tropical Sericulture Practices (Feb. 17–23, Bangalore)*, 61.

Horie, Y. and Watanabe, H. (1983) Effect of various kinds of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in the silkworm, *Bombyx mori*. *J. Insect Physiol.* **29(2)**: 187–199.

House, H. L. (1974) In: *The Physiology of Insecta*, 2nd edition, Vol. 5. Rockstein, M. (Editor). Academic Press: New York, 3–4.

Ismail, S. M. and Dutta-Gupta, A. (1988) Juvenile hormore inhibits the synthesis of major larval haemolymph proteins in rice moth *Corcyra cephalonica*, *Biochem. Int.* **17**: 1093–1098.

Izumi, S., Fujie, J., Yamada, S. and Tomino, S. (1981) Molecular properties and biosynthesis of major plasma proteins in *Bombyx mori*, *Biochem. Biophys. Acta.* **670**: 227–229.

Janarthanan, S. (1995) The nutritional quantity of mulberry leaves in relation to their age and variety and its effect on silkworm *Bombyx mori*, Bharathidasan University, Thiruchirapalli, T.N. India, submitted

Janarthanan, S., Sankar, S., Krishnan, M. and Ignacimuthu, S. (1999) Nutritional and hormonal effects on storage proteins in the silkworm *Bombyx mori*, L., *J. Biosci.* **24(1)**: 53–57.

Jayswal, K. P., Singh, T. and Subba Rao, G. (1991) Effect of female pupal weight and fecundity in *Bombyx mori*, *Indian J. Seri.* **30(2)**: 141–143.

Keshan, B. and Ray, A. K. (2000) Estradiol-17&β; *Bombyx mori* possible significant and its effect on silk production, *J. Insect Physiol.* **46**: 1061–1068.

Kotikal, Y. K., Reddy, D. N. R., Prabu, A. S., Bhat, G. G. and Pushpalatha, S. (1989) Relationship between pupal size and egg production in Eri Silkworm, *Samia cynthia ricini* (Lepidoptera: Saturniidae), *Indian. J. Seric.* **28**: 80–82.

Krishnan, M., Subburathinam, K. M. and Janarthanan, S. (1995) Effect of hydrolysed protein (P–Soyatose) on haemolymph protein profile, larval and pupal characters of the silkworm, *Bombyx mori* L, (Lepidoptera: Bombycidae), *Sericologia.* **35(2)**: 227–235.

Krishnaswami, S., Kasiviswanathan, K. and Sitarana Iyengar, M. N. (1970) Effect of feeding leaves grown under different systems of mulberry cultivation on the silkworm cocoon crop, *Indian J. Seric.* **2(1)**: 53–58.

Kumararaja, S., Vijayaraghavan, S. and Krishnaswami, S. (1972) Studies on fortification of mulberry leaves for feeding silkworms, *Indian J. Seric.* **11**: 68–72.

Laemmli, V. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature, London* **227**: 680–685.

Levenbook, L. (1985) Insect storage proteins, In: *Comprehensive Insect Biochemistry, Physiology and Pharmacology*, Gilbert, L. I. and Kerkut, G. (Editors). Pergamon Press: Oxford, **10**: 307–346.

Madhavan, S., Baskaran, K., Sironmani, A. and Pandian, T. J. (1984) Studies on the utilisation of single cell protein by the silkworm, *Bombyx mori*, *Entomol. Exp. Appl.* **36**: 61–68.

Mine, E., Izumi, S., Kutsuki, M. and Tomino, S. (1983) Developmental and sex dependent regulation of storage protein synthesis in the silkworm, *Bombyx mori*, *Dev. Biol.* **97**: 329–337.

Mukherjee, P. K., Roy, A. K. and Chakraborty, N. G. (1983) Studies on the effect of pupal weight on fecundity and hatching ability of multivoltine silkworm race *Bombyx mori* L., *National Seminar on silk Research and Development (Mar. 10–13, Bangalore)*, 28.

Nagalakshmamma, K., Gowda, B. L. V. and Develah, M. C. (1998) Relationship of pupal weight on fecundity and hatching in Eri moths *Samia Cynthia ricini* Biosdual, *Int. Congress on Trop. Seri. Practices (Feb. 17–23, Bangalore)*, 56.

Nanjundappa, T. and Varghese, T. J. (1989) Effect of diethylstilbestrol on growth and food conversion of common carp *Cyprinus carpio* (Linn.), *Proc. Indian Acad. Sci. (Anim. Sci.)* **98(2)**: 85–88.

Nijhout, H. F. (1975) A threshold size for metamorphosis in the tobacco hornworm, *Manduca sexta* (L), *Biol. Bull. Mar. Biol. Labwoods Hole.* **149**: 214–225.

Nirmala, A. R. C. and Pandian, T. J. (1983) Effect of Steroid injection on food utilisation in *Channa stratus*, *Proc. Indian Acad. Sci. (Anim. Sci.)* **92**: 221–229.

Rahman, M. N., Mondal, K. A. M. S. H. and Khalequzzaman, M. (1978) Oviposition studies in the silkworm, *Bombyx mori* L. race Nistari C/40 (Lepidoptera: Bombycidae), *J. Zool. Bangladesh* **6**: 1–6.

Sakai, N., Mori, S., Izumi, S., Fukushima, K. H., Ogura, J., Maekawa, H. and Tomino, S. (1988) Structures and expression of mRNA coding for major plasma proteins of *Bombyx mori*, *Biochem. Biophys. Acta.* **949**: 224–232.

Shaheen, A., Trag, A. R., Nabi, G. H. and Ahamad, F. (1992) Correlation between female pupal weight and fecundity in bivoltine silkworm, *Bombyx mori* L, *Entomon.* **17(1&2)**: 109–111.

Siddique, A. A., Debnath, A. K. and Sengupta, K. (1985) Variability and correlative studies of shell weight and their contributing traits in *Antheraea mylitta* D, *Sericologia* **25(1)**: 45–50.

Singh, Y. R. and Prasad, B. (1987) Correlation among body weight and fecundity in Eri silkworm, *Philosomia ricini* Hutt. (Lepidoptera: Saturniidae), *Sericologia* **27(1)**: 49–50.

Swevers, L., Lambert, J. G. D. and De Loof, A. (1991) Synthesis and metabolism of vertebrate-type steroids by tissues of insects. A critical evaluation, *Experentia* **47(2)**: 687–698.

Tojo, S, Nagata, M. and Kobayashi, M. (1980) Storage proteins in the silkworm, *Bombyx mori*, *Insect Biochem.* **10**: 289–303.

Tojo, S., Kiguchi, K. and Kimura, S. (1981) Hormonal control of storage protein synthesis and uptake by the fat body in the silkworm, *Bombyx mori*, *J. Insect Physiol.* **27**: 491–497.

Tojo, S., Morita, M., Agui, N. and Hiruma, K. (1985) Hormonal regulations of phase polymorphism and storage protein fluctuation in the common cutworm, *Spodopteron litura*, *J. Insect Physiol.* **31**: 282–292.

Vanishree, V., Nirmala, X. and Krishnan, M. (1999) Differential synthesis of storage proteins by various fat body tissues during development of female silkworm, *Bombyx mori*, *SAAS BULL: Biochem Biotech.* **12**: 69–89.

Venkata Rami Reddy, K., Magadum, S. B., Meera, V. and Dutta, R. K. (1994) Metabolic significance of vertebrate-type steroids in silkworm, *Bombyx mori* L, A. Review, *Indian J. Seric.* **33(1)**: 6–11.

Zar, J. H. (1999) In: *Biostatistical Analysis*, 4th Edition. Ryu, T. (Editor). Prentices Hall International, INC: NJ, 123.

Zhu, J., Indrasith, L. S. and Yamashita, O. (1986) Characterisation of Vitellin egg specific protein and 30 kDa protein from *Bombyx* eggs and their fates during oogenesis and embryogenesis, *Biochem. Biophys. Acta.* **882**: 427–437.

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Redescription of adult *Acalitus hibisci* Mondal & Chakrabarti (Acari: Eriophyoidea) and description of its different developmental stages

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ABSTRACT: *Acalitus hibisci* is a pouch gall forming mite which infests *Hibiscus vitifolius*, a wild shrub. The species is redescribed in the light of SEM studies. Different developmental stages of this mite are also described.

KEYWORDS: *Acalitus hibisci*, Eriophyoidea, Acari, Redescription, West Bengal, India

INTRODUCTION

Mondal and Chakrabarti (1982) described a new species, *Acalitus hibisci* collected from leaves of *Hibiscus vitifolius*, a wild shrub form Kalyani, India. Recently, this species was studied under SEM. Some features that were detected form SEM studies are described here. The morphological characters of different developmental stages of the mite are also described here. All measures are expressed in μm.

MATERIAL AND METHODS

Nuzzaci and Volvas (1976) described the sample preparation of live specimens of eriophyid mites for SEM studies. Nuzzaci *et al.* (1991) further illustrated some preparation techniques of preserved eriophyids, specially the dried and mummified ones. Alberti and Nuzzaci (1996) fixed the whole leaf containing mites during their normal activities by plunging the leaf into 10% acrolene for four hours and then the same were placed in 6% gulteraldehyde for several hours. After dehydration in graded acetone the leaves are cut into sections and critical point dried. In our present study, the above method was modified for live specimens. Live specimens (female)

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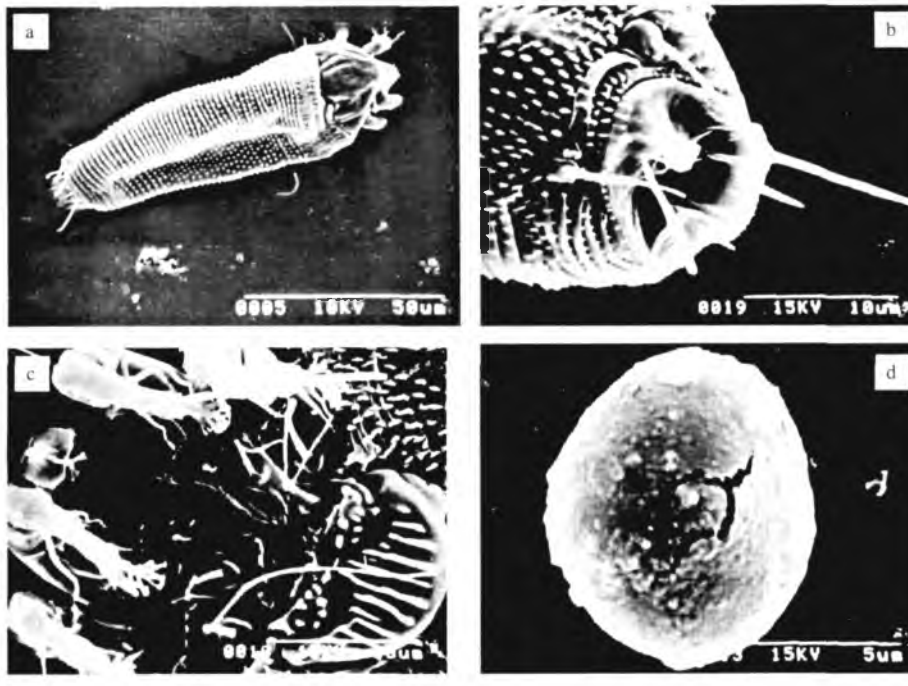


PHOTOGRAPHS 1–3: *Acalitus hibisci* Mondal and Chakrabarti (Scanning electron Micrographs): Photo 1. Dorsal view of adult, Photo 2. Accessory seta (ventro-caudal region), Photo 3. Coxae and epigynium, Photo 4. Egg.

were collected by a fine needle from the gall of the leaves of *Hibiscus vitifolius* and place singly on the electron microscopic stub. Chemical fixation, cleaning and drying techniques were not applied here. The specimens were directly gold-coated and then placed within the vacuum chamber of the SEM and photographs were taken.

For Phase Contrast Microscopic studies the mites of different stages are collected and permanent slides are prepared by 'Hoyers medium' (Baker and Wharton, 1952) and observed under microscope.

Description

Female (Photographs 1–3)

Following additional characters/discrepancies have been noticed from the description provided by Mondal and Chakrabarti (1982).

Body 145-152 long (Photo 1) gnathosoma (Fig. 1) having submedian lines two in number, first one runs posteriorly and divergently upto 0.6 parts of prodorsal shield, then bifurcated, inner branch meets the median line near the rear shield margin and the outer branch meets the rear shield margin leaving the dorsal tubercles in between the

two forks. Accessory seta present (Photo 2). Gential coverflap (Photo 3) with about 12 longitudinal scorings and a few granules.

Male: (Figs 2–5)

Body (Fig. 2) 127-140 long, 41-42 wide, gnathosoma 11 long, subapical pedipalp tarsal seta 6-7 long; dorsal tubercle 16-17 apart, scapular seta 16-18 long; leg I (Fig. 3) 21-23 long from trochanter base, femur 5-6 long with a small basiventral femoral seta 4-6 long, antaxial genual seta 15-17 long, tibia 2-3 long with two antaxial fastigial tarsal seta each 14-15 long, tarsal solenidion 5-7 long, tarsal empodium simple 5 rayed; leg II (Fig. 4) 20-22 long from trochanter base, genu 3-4 long with seta 10 long, tibia 3 long, tarsus 5-7 long, other characters same as leg I; anterolateral seta on coxisternum I slightly on the level of coxa I approximation, proximal seta on coxisternum II.

Opisthosoma with about 54-58 annuli, seta c2 20-21 long on about ventral annulus 10; seta d 34-35 long on about annulus 23; seta e 4 long on about ventral annulus 34; seta f 14 long on about ventral annulus 57; seta h1 3-4 long, seta h2 26-31 long; epigynium (Fig. 5) 14-15 wide and 9-11 long, with a grooved structure and a pair of papilla, proximal seta on coxistrernum III 4-4.6 long, 11.6-12 apart.

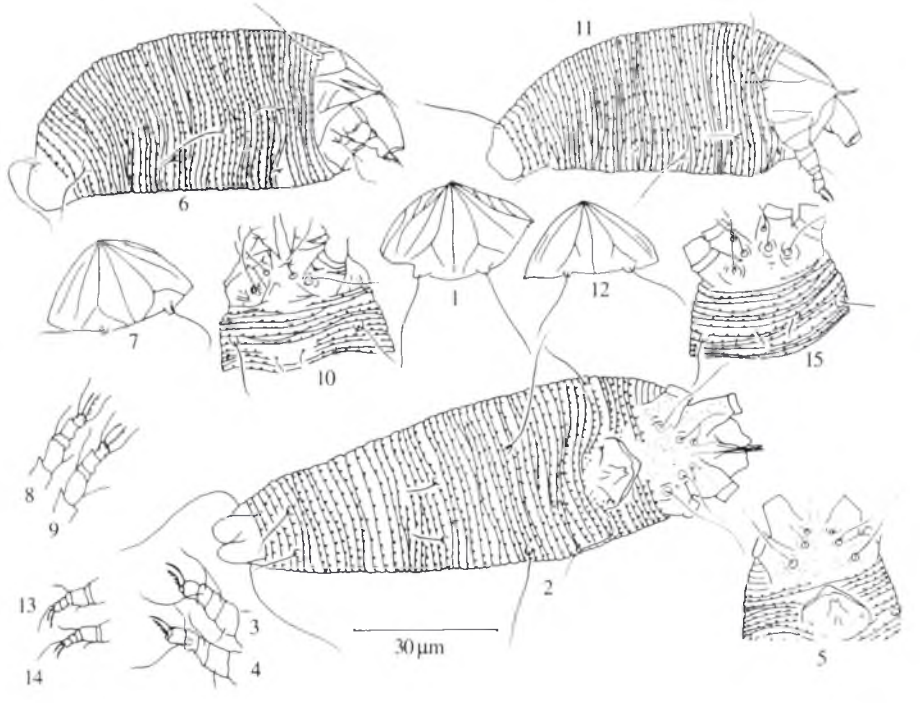
Deutonymph (Figs 6–10)

Body (Fig. 6) 98-107 long, 36-37 wide, gnathosoma 9 long, subapical pedipalp tarsal seta 5-7 long; dorsal tubercle (Fig. 7) 14-15 apart, scapular seta 16-18 long; leg I (Fig. 8) 14-15 long from trochanter base, femur 4-5 long with a small basiventral femoral seta 3 long, genu 2 long, antaxial genual seta 8-11 long, tibia 1-2 long with two antaxial fastigial tarsal seta each 13-14 long, tarsal solenidion 5 long; leg II (Fig. 9) 14-16 long from trochanter base, genu 2 long with seta 9-11 long; other characters same as leg I.

Opisthosoma with about 56 dorsal annuli and almost equal number of ventral annuli. Seta c2 10-12 long on about ventral annulus 9; seta d 16-17 long on about annulus 21; seta e2 long on about ventral annulus 32; seta f 11 long on about ventral annulus 49; seta h1 2-3 long, seta h2 29-30 long; epigynium (Fig. 10) not developed but a smooth 8-9 wide and 2-3 long area appeared in between the proximal seta on coxiosternum III on the ventral thanosome, proximal seta on coxsternum III 1-2 long, 7-8 apart.

Protonymph (Figs 11–15)

Body (Fig. 11) 82-84 long, 37-39 wide, gnathosoma 8-9 long, subapical pedipalp tarsal seta 4-5 long; dorsal tubercle (Fig. 12) 11-12 apart, scapular seta 16-18 long; leg I (Fig. 13) 14-15 long from trochanter base, femur 4-5 long with a small basiventral femoral seta 2-3 long, genu 2 long, antaxial genual seta 8-9 long, tibia 1-2 long, tarsus 4-5 long, with two antaxial fastigial tarsal seta each 14-15 long, tarsal solenidion 4-5 long; leg II (Fig. 14) 13-14 long from trochanter base, genu 2-3 long with antaxial genual seta 6-7 long, tibia 1-2 long, tarsus 3-4 long, other characters same as leg I.



FIGURES 1–15: *Acalitus hibisci* Mondal and Chakrabarti: 1. Prodorsal shield of adult, 2. Ventral view of the male, 3. Leg I of male, 4. Leg II of male, 5. Epygynium of male, 6. Lateral view of deutonymph, 7. Prodorsal shield of deutonymph, 8. Leg I of deutonymph, 9. Leg II of deutonymph, 10. Ventral view of thanosome of deutonymph, 11. Lateral view of protonymph, 12. Prodorsal shield of protonymph, 13. Leg I of protonymph, 14. Leg II of protonymph, 15. Ventral view of protonymph.

Opisthosoma with about 51 dorsal annuli and almost equal number of ventral annuli, seta d 16-18 long on about annulus 19; seta e 1-2 long on about ventral annulus 28; seta f 10-11 long on about ventral annulus 45; seta h2 25-27 long; epigynium (Fig. 15) not developed but minute proximal seta on conxisternum III present on ventral thanosome, proximal seta on coxisternum III 1-2 long, 6-7 apart. Other characters as in deutonymph.

Egg

The egg (Photo 4) measuring 36.313 ± 0.776 in diameter, transparent and white in colour, laid singly and glued to surface of the leaves within the gall.

Material studied

Type material: Holotype: female on slide (No. 161/33/77), India: West Bengal: Nadia, Kalyani, 11.xii.1977 from *Hibiscus vitifolius* L. (Malvaceae) coll. S. Chakrabarti. Paratypes: females on 3 slides (Nos 162/75/80 to 164/75/80), collected on 4.iii.1980

from the same plant and locality by S. Mondal. Additional materials studied: many females, a few males and nymphal instars: India: West Bengal: Kalyani, 27.iii.99, 12.v.99, 27.vii.99, 12.x.99, 27.xii.99 from *Hibiscus vitifolius* (Malvaceae), coll. Saswati Chakrabarti; many females, few males and nymphal instars: India: West Bengal: Shyamnagar, 27.iv.99, 12.ix.99, 27.1.2000, 12.2.2000, coll. Saswati Chakrabarti.

Relation to the host

The species is a pouch gall forming mite and remains generally on the lower surfaces of the leaves. But during heavy infestation a few galls are found also on the upper surface of the leaves.

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REFERENCES

Alberti, G. and Nuzzaci, G. (1996) SEM and TEM techniques, In: *'Eriophyid mite—Their Biology, Natural Enemies and Control*, Lindquist, E. E. *et al.* (Editor). Elsevier Science: 399–410.

Baker, E. W. and Wharton, G. W. (1952) *An Introduction to Acarology*, The Macmillan: New York, 465.

Mondal, S. and Chakrabarti, S. (1982) Studies on the eriophyid mites (Acarina: Eriophyoidea) of India. XI. Descriptions of three new species from West Bengal, *Entomon.* **7(3)**: 361–366.

Nuzzaci, G. and Volvas, N. (1976) Osservazioni dei caratteri tassinomici degli Eriofidi al microcsopio elettronico a scansione, *Atti 1 Congr. Naz. It. Entomol., Portici-Sorrento* **1**: 117–122.

Nuzzaci, G., Lillo de E, and Mariani, G. R. (1991) Scanning microscopy in Acarology: a new technique for preparation of eriophyids preserved in different ways, *Boll. Soc. Ent. Ital., Genova* **123(1)**: 3–8.

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Distribution patterns of style lengths in *Ficus* and the ovipositor size of their chalcid pollinators (Agaonidae: Hymenoptera): an analytical study

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ABSTRACT: An analysis of style length distribution in different species of *Ficus* with respect to the ovipositor lengths of their corresponding agaonid pollinaotrs showed a positive unimodal pattern in monoecious figs and a bimodal one in gynodioecious species. These two patterns were evolved to suit the ovipositor lengths of their respective pollinators. Four species of monoecious figs namely, *Ficus religiosa*, *F. drupaceae*, *F. racemosa* and *F. benghalensis* pollinated respectively by *Platyscapa quadraticeps* Mayr, *Eupristina belgaumensis* Joseph, *Ceratosolen fusciceps* Mayr and *Eupristina masoni* Saunders; and two gynodioecious species *viz.*, *F. hispida* and *F. exasperata* pollinated by *Ceratosolen solmsi marchali* Mayr and *Kradibia gestroi* Grandi respectively were selected for this study. The significance of this relationship in insect-plant mutualism is discussed.

KEYWORDS: *Ficus spp.*, agaonid pollinators, style length, ovipositor, distribution patterns

INTRODUCTION

The relationship between fig trees (*Ficus*, Moraceae) and their pollinating chalcid wasps (Agaoninae: Agaonidae) is one of the best known examples of plant-animal coevolved mutualism (Corner, 1967; Galil, 1973; Wiebes, 1979; Anstett *et al.*, 1997). Of the 750 known species of figs found world wide, except in one or two cases, each one is exclusively pollinated by its own specific wasp (Wiebes, 1993). About half of the known *Ficus* species are monoecious,the other half being gynodioecious. The monoecious figs bear two distinct kinds of female flowers: those with long styles producing seeds, as their ovaries are beyond the reach of the wasp's ovipositor; and those with short styles into which the wasps can lay eggs, thus transforming them to ovarian galls favouring their own development (Galil and Eisikowitch, 1969; Joseph and Abdurahiman, 1984). In gynodioecious species two kinds of trees are found: the male (hermaphrodite) tree with figs that contain both male and female flowers, and the female tree with syconia that bears female flowers only. Recent studies show that

TABLE 1. List of *Ficus* species studied with their pollinator wasp

Ficus species	Pollinator wasps (Agaoninae: Agaonidae)
Monoecious	
Ficus drupaceae Thunb	*Eupristina belgaumensis* Joseph
F. racemosa L	*Ceratosolen fusciceps* Mayr
F. religiosa L	*Platyscapa quadraticeps* mayr
F. benghalensis L	*Eupristina masoni* Saunders
Gynodioecious	
Ficus hispida L	*Ceratosolen solmsi marchali* Mayr
F. exasperata Vahl	*Kradibia gestroi* Grandi

in monoecious fig species there is a unimodal distribution of style lengths (Van Noort *et al.*, 1989; Kathuria *et al.*, 1995, 1997; Ganeshaiah *et al.*, 1995, 1997), whereas in gynodioecious species there is uniformly a bimodal distribution. In the present work, based on the data gathered on the length of styles of a few species of *Ficus* belonging to the two categories mentioned above, with respect to the ovipositor lengths of the corresponding pollinating wasps, the distribution patterns were analysed in an attempt to correlate these findings to the mutualism involved.

MATERIALS AND METHODS

Figs were collected from six species of *Ficus* from different localities of Kozhikode and Malappuram districts of Kerala. The required data were gathered from four monoecious and two dioecious species of *Ficus* (Table 1). At least two trees of each category were selected for regular observations. For measuring the style length, 30 flowers were randomly collected from each B-phase syconium according to the method followed by Galil and Eisikowitch (1969). The tepals were removed and style lengths were measured from the stigma to the point of attachment to the ovary, to the nearest 0.01 mm, under a microscope. The figs of D-phase collected from the field were kept in jars covered with muslin cloth. The adult wasps that emerged were collected and preserved in 70% alcohol. A minimum of 30 female wasps were randomly selected from each species to measure the length of the ovipositor. The ovipositor was separated from its enveloping sheaths and measured from the point of attachment to the gaster till the tip, to the nearest 0.01 mm. Kolmogorow-Smirnow test was employed to test the fitness of the style length.

RESULTS

The style lengths of four species of monoecious figs showed a continuous distribution, there was no indication of bimodality or existence of discrete classes of short and long styled flowers (Table 2). This showed that style lengths of monoecious figs seem to exhibit a unimodal distribution (Figs 1, 2, 3 and 4). However, it is possible to identify

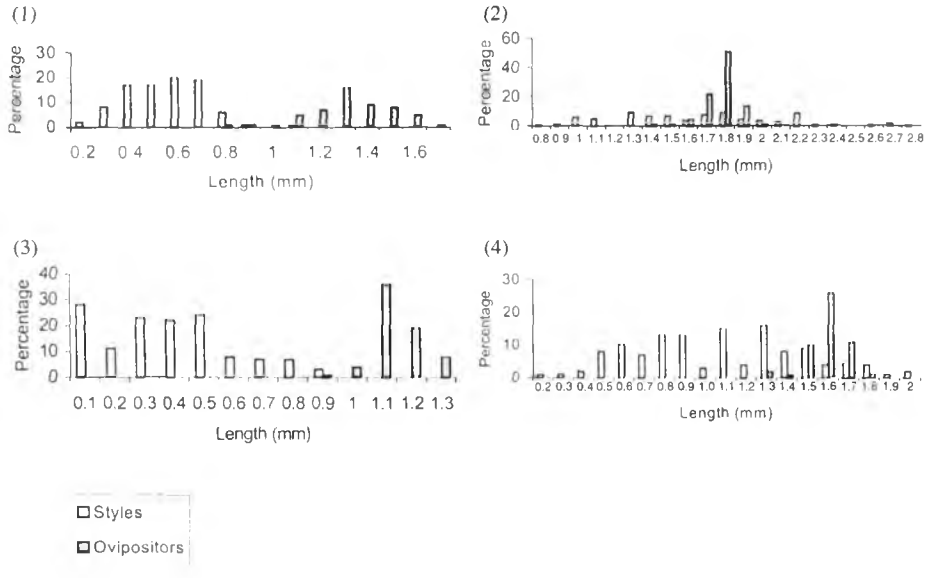
TABLE 2. Mean style lengths of four monoecious and two gynodioecious fig species and the ovipositor lengths of their pollinator wasps

Ficus species with the wasp species in brackets	Style length and ovipositor length (mm)				
	n	Mean	SD	CV(%)	Percentage of short styled flower
Monoecious					
Ficus drupaceae	91	0.6012	0.169	28.11	100
(Eupristina belgaumensis)	54	1.364	0.182	13.34	
F.racemosa	86	1.716	0.468	27.27	62.7
(Ceratosolen fusciceps)	97	1.822	0.099	5.43	
F.religiosa	132	0.420	0.220	52.38	100
(Platyscapa quadraticeps)	68	1.184	0.082	6.92	
F. benghalensis	126	1.122	0.400	35.65	97.6
(Eupristina masoni)	51	1.841	0.132	7.17	
Gynodioecious					
F. hispida-Gall fig	100	0.382	0.082	21.46	100
(Ceratosolen solmosi marchali)	102	0.628	0.032	5.09	
F.hispida-seed fig	95	1.118	0.176	15.74	0
F. exasperata	54	0.761	0.142	18.65	79.6
Gall fig *(Kradibia gestroi)*	44	0.914	0.052	5.68	
F.exasperata seed fig	50	1.426	0.183	12.83	0

short and long styled flowers relative to the length of the ovipositor of respective pollinating wasp. It indicates the fact that in *Ficus religiosa* and *F. drupaceae*, 100% of the styles in the syconium were shorter than the mean ovipositor length of their respective wasps, *Platyscapa quadraticeps* Mayr and *Eupristina belgaumensis* Joseph. In the case of *Ficus racemosa* and *F. benghalensis*, which are pollinated by *Ceratosolen fusciceps* Mayr and *Eupristina masoni* Saunders, the percentage of the short styled flowers in the syconia were 62 and 98 respectively (Table 2).

In gynodiecious figs *viz.*, *Ficus hispida* and *F. exasperata*, pollinated respectively by *Ceratosolen solmsi marchali* Mayr and *Kradibia gestroi* Grandi a bimodality of the style lengths exist in hermaphroditic gall figs and female seed figs (Figs 9 and 10). When the style length distribution of gall fig syconia of *F. hispida* and *F. exasperata* were examined, it was noted that in the former 100% and in the latter 80% flowers were short styled* so that the pollinator wasps have access to almost all flowers in a syconium (Table 2, Figs 5 and 7); whereas females syconia (seed fig) of both these gynodioecious species carry long styled flowers and thus have zero fitness to the

*Flowers with style lengths smaller than the mean ovipositor lengths were considered short styled.

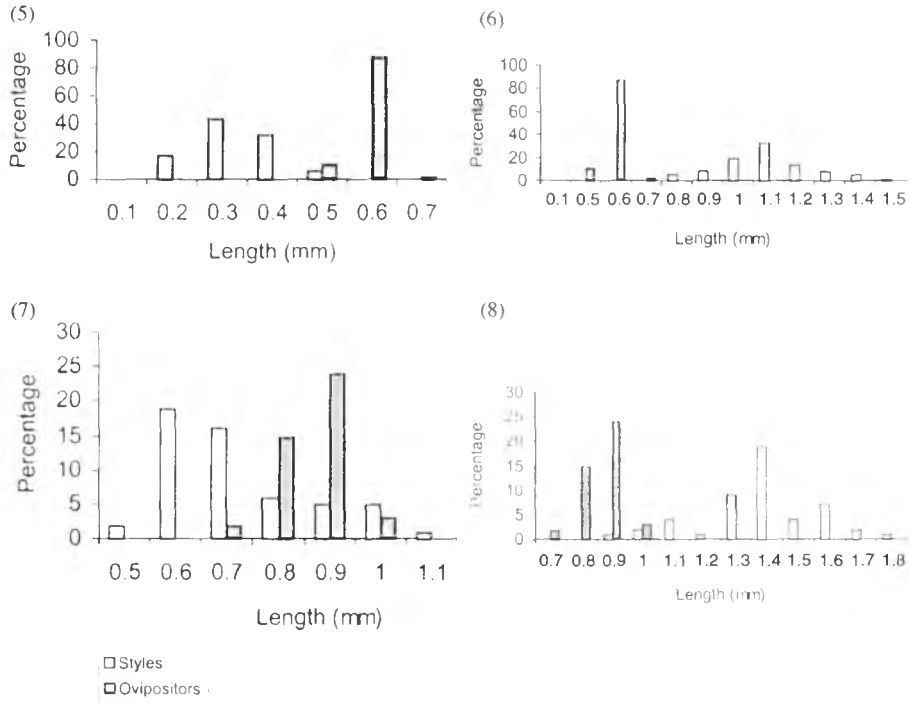


FIGURES 1–4: Distribution patterns of style lengths of four monoecious *Ficus* species and ovipositor lengths of their pollinating wasps. 1. Style length of *Ficus drupaceae* and ovipositor length of *Eupristina belgaumensis*. 2. Style length of *F. racemosa* and ovipositor length of *Ceratosolen fusciceps*. 3. Style length of *F. religiosa* and the ovipositor length of *Platyscapa quadraticeps*. 4. Style length of *F. benghalensis* and the ovipositor length of *Eupristina masoni*.

wasps, i.e., the pollinators have absolutely no access to any ovary in the syconium (Figs 6 and 8) resulting in 100% seed production. The coefficients of variation in the distribution of style lengths especially of all monoecious species of figs were two to five times more than that in the ovipositor length of their pollinator wasps (Table 2).

DISCUSSION

The comparative analyses of style length distribution of monoecious and gynodioecious fig species provide valuable insights into the origin and evolution of plant-wasp mutualism. In monoecious fig syconia, the pollinators have long ovipositors which gain access to many more ovaries than they actually oviposit in (Table 2). Bronstein (1988), Nefdt and Compton (1996), Ganeshaiah *et al.* (1995, 1997) and Kathuria *et al.* (1995, 1997) have made same observations in different species of *Ficus* and their respective pollinators. The style lengths of the four monoecious fig species examined had showed a continuous distribution rather than the expected bimodal distribution (Figs 1–4). This observation is contradictory to the statement given by Janzen (1979); Galil and Eisikowitch (1971); Johri and Konar (1956); Hill (1967) and Ramirez (1974). Murray (1985) argued that half of the styles in the monoecious figs are longer than the ovipositor of their respective pollinators. Ganeshaiah *et al.* (1995) studied 18 fig wasp species and found that their ovipositors are accordingly



FIGURES 5–8: Distribution patterns of style lengths of two gynodioecious *Ficus* species and ovipositor lengths of their pollinating wasps. 5. Style length of *F. hispida* (gall fig) and the ovipositor length of *Ceratosolen solmsi marchali.* 6. Style length of *F. hispida* (seed fig) and the ovipositor length of *C. solmsi marchali.* 7. Style length of *F. exasperata* (gall fig) and the ovipositor length of *Kradibia gestroi.* 8. Style length of *F. exasperata* (seed fig) and the ovipositor length of *Kradibia gestroi.*

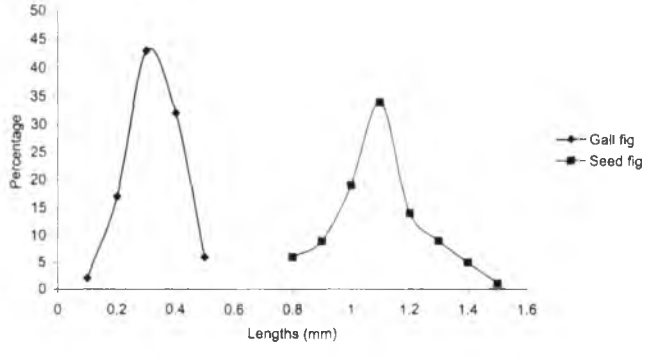


FIGURE 9. Distribution of style lengths of *F. hispida* (gall fig and seed fig)

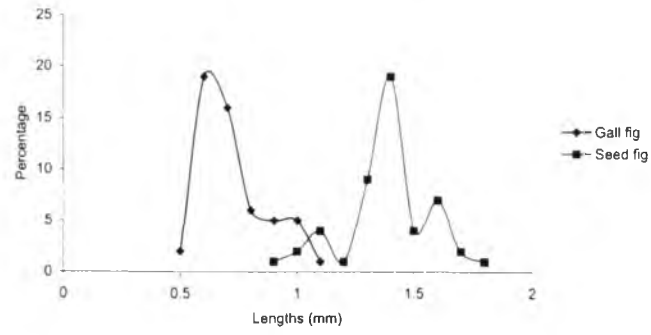


FIGURE 10. Distribution of style lengths of *F. exasperata* (gall fig and seed fig)

optimised being capable of using 80–90% of the flowers in the syconia of their host plant.

The conditions that dioecious fig should fulfill are quiet different from those for a monoecious fig. The observations from *Ficus hispida* and *F. exasperata* confirmed that the sexual functions are effected by two traits, that is the absence of functional anthers in syconia of female trees and a difference in style lengths between female flowers within the hermaphroditic and female syconia. Figs 9 and 10 show the style length difference between hermaphroditic and female *F. hispida* and *F. exasperata* species, where the bimodal distribution of style length is clear. It is obvious that the hermaphroditic syconia of dioecious fig species are used for male function by their respective pollinators, exploiting nearly 100% of the female flowers in the syconia (Figs 5 and 7), while the seed fig syconia is precluding pollinators' ovipositon by their long style length (Figs 6 and 8). Moreover, the female syconia is used in female function by producing seeds only. Kjellberg *et al.* (1987) made the same observation in *Ficus carica*, and Patel (1996) in *Ficus hispida* and *F. exasperata*, but their findings on the stability of symbiosis and coevolved mutualism among dioecious figs and their pollinators were based on seasonal climatic studies and not depended on the floral character and ovipositor length.

The coefficients of variation in the distribution of style lengths with the ovipositor length of their pollinator wasps were two to five times more except in *F. religiosa* where it exceeds seven times. According to Bronstein (1992) the distribution of style lengths seems to be a proximate consequence of the dense packing of flower priomordia and competitive style growth during early development within the syconium and that the styles of different length have similar cell numbers. Evolutionarily it may be an indirect effect of selection for increased numbers of flowers per syconium. The evolutionary relationships within each species of *Ficus* and pollinator wasp is greatly influenced by the morphological traits specially designed for the accomplishment of oviposition and pollination, The fig-pollinator interaction is the best model system used for understanding mutualism. As stated by Bronstein (1992), the reciprocal benefits merge within mutualists as a result of reciprocal exploitation. For ensuring the

stability of symbiosis, the fig tree and pollinator insects have adopted certain structural preadaptive measures; like, the nature of ostiole which is instrumental in checking and regulating the entry of pollinating wasps, special morphological features of the female insects that facilitate their entry into the syconium and the relative lengths of ovipositor and style for effectively controlling oviposition. The evolutionary conflicts that exist between figs and fig wasp as mutualists are mainly over issues concerned with female flower exploitation.

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REFERENCES

Anstett, M. C., Mc Key, and Kjellberg, F. (1997) Figs and fig pollinators: evolutionary conflicts in a coevolved mutualism, *Tree* **12**: 94–99.

Bronstein, J. L. (1988) Mutualism, antagonism and the fig-pollinator interaction, *Ecology* **69**: 1298–1302.

Bronstein, J. L. (1992) *Insect-Plant Interactions*, Bernays, E. (Editor). CRC Press: Boca Raton, FL.

Corner, E. J. H. (1967) *Ficus* in the Solomen Islands and its bearing on the post-Jurassic history of Melanesia, *Philos. Trans. Roy. Soc. London (B)* **253**: 23–159.

Galil, J. (1973) Pollination in dioecious figs. Pollination of *Ficus fistulosa* by *Ceratosolen hewitti*, *Gdn's. Bull. Singapore* **26**: 303–311.

Galil, J. and Eisikowitch, D. (1969) Further studies on the pollination ecology of *Ficus sycomorus* L. (Hymenoptera, Chalcidoidea, Agaonidae), *Tijdschr. Ent.* **112**: 1–13.

Galil, J. and Eisikowitch, D. (1971) Studies on mutualistic symbiosis between syconia and sycophilious wasps in monoecious figs, *New Phytol.* **68**: 1243–1244.

Ganeshaiah, K. N., Kathuria, P., Umashaankar, R. and Vasudeva, R. (1995) Evolution of style length variability in figs and optimization of ovipositor length in their pollinator wasps: a coevolutionary model, *J. Genet.* **74**: 25–39.

Ganeshaiah, K. N., Kathuria, P. and Umashaankar, R. (1997) *Ecology and Evolution of Plant Feeding Insects in Natural and Man Made Environments*, Raman, A (Editor). International Scientific Publications: New Delhi, 169–182.

Hill, D. S. (1967) Fig wasps (Chalcidoidea) of Hong Kong. 1. Agaonidae, *Zool. Verh. Leiden* **89**: 1–55.

Janzen, D. H. (1979) How many babies do figs pay for more babies? *Biotropica* **11**: 48–50.

Johri, B. M. and Konar, R. N. (1956) The floral morphology and embryology of *Ficus religiosa* Linn, *Phytomorphology* **6**: 97–111.

Joseph, K. J. and Abdurahiman, U. C. (1984) Agaonid-*Ficus* interactions with special reference to pollination ethology, *Proc. Indian Acad. Sci. (Anim Sci.)* **93**: 365–372.

Kathuria, P., Ganeshaiah, K. N., Umashaankar, R. and Vasudeva, R. (1995) Is there dimorphism for style length in monoecious figs? *Curr. Sci.* **68**: 1047–1049.

Kathuria, P., Ganeshaiah, K. N. and Vasudeva, R. (1997) *Ecology and Evolution of Plant Feeding Insects in Natural and Man Made Environments*, Raman, A (Editor). International Scientific Publications: New Delhi, 183–195.

Kjellberg, F., Gouyn, P. H., Ibrahim, M., Raymond, M. and Valdeyron, G. (1987) The stability of symbiosis between dioecious figs and their pollinators: a study of *Ficus carica* L. and *Blastophaga psenes* L., *Evolution* **41**: 693–703.

Murray, M. G. (1985) Figs (*Ficus* spp.)and fig wasps (Chalcidoidea, Agaonidae): hypothesis for an ancient symbiosis, **26**: 69–81.

Nefdt, R. J. C. and Compton, S. G. (1996) Regulation of seed and pollinator production in the fig–fig wasp mutualism, *J. Anim. Ecol.* **65**: 170–182.

Patel, A. (1996) Variation in a mutualism: phenology and the maintenance of gynodioecy in two Indian fig species, *J. Ecol.* **84**: 667–680.

Ramirez, B. W. (1974) Coevolution of *Ficus* and Agaonidae, *Ann. Missouri. Bot. Gdn.* **61**: 770–780.

Van Noort, S., Ware, A. B. and Compton, S. G. (1989) Pollinator-specific volatile attractants released from the figs of *Ficus burtt-davyi*, *S. Afr. J. Sc.* **85**: 323–324.

Wiebes, J. T. (1979) Coevolution of figs and their insect pollinators, *Ann. Rev. Ecol. Syst.* **10**: 1–12.

Wiebes, J. T. (1993) Agaonidae (Hymenoptera: Chalcidoidea) and *Ficus* (Moraceae): fig wasps and their figs XI. (Blastophaga), *Proc. Kon. Ned. Akad. V. Wetensch* **96**: 347–367.

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Persistence of indigenous *Bacillus thuringiensis* var *galleriae* (Spicturin®) on cauliflower against *Plutella xylostella* L.

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ABSTRACT: The indigenous liquid *B.t.* formulation, Spicturin® contains both spores and toxins of *Bacillus thuringiensis* var *galleriae*. The pot culture experiments were conducted for two seasons to evaluate the persistent period (PP), persistence toxicity (PT) and persistence toxicity index (PTI) of the Spicturin (4, 3 and 2 ml/l) against first and third instar larvae of *P. xylostella* on cauliflower. The results of the persistence study indicated that the PP was found to be higher for Spicturin @ 4 ml/l (96 hrs) followed by Spicturin @ 3 and 2 ml/l (72 hrs) on cauliflower against first and third instar for Spicturin @ 4 ml/l followed by Spicturin @ 3 and 2 ml/l on cauliflower against *P. xylostella*. The PP, PT and PTI were found to be increased when the surfactant, Teepol (1 ml/l) was added to Spicturin irrespective of the doses of the formulation and these values were decreased as the larval instars advanced irrespective of the doses of Spicturin and seasons. The persistence of Spicturin was higher in winter season when compare to summer season. This may be due to the degradation of toxic protein in the formulation due to high temperature.

KEYWORDS: *Bacillus thuringiensis* var *galleriae*, persistence, *Plutella xylostella*, cauliflower

B.t. is one of the most widely used biocides for insect pest management, particularly the lepidopterous pests. The pathogenicity and mode of action of *B.t.* has been well documented by Beegle and Yamamoto (1992) and Knowles (1994). With a growing concern for a clean environment, more and more emphasis is being focussed on biopesticides particularly the *B.t.* based products. The pesticide industry is coming out with newer *B.t.* commercial formulations in a big way. *B.t.* is the most extensively studied microbial agent, the control potential of which has been investigated on a number of insect species in different countries. In India, it is used to the extent of about 30 tonnes annually (Gujar *et al.*, 1998).

*Corresponding author

The insecticidal action of isolates of *B.t.* are complex. The relative activity of each isolate against different insect species i.e. its 'Spectrum of activity' arises partly from the combined effects of the potencies of the varying components of the different insecticidal materials which they produce. Delta endotoxins from different isolates of *B.t.* can kill different insects species or differ in their degree of activity towards them. The present investigations were made on the persistence of SPICTURIN®, the indigenous liquid *B.t.* formulation based on *Bacillus thuringiensis* var. *galleriae* on cauliflower.

With the slight modifications in the methodology of Pinnock *et al.* (1971), the persistence of Spicturin (*Bacillus thuringiensis* var *galleriae*) on cauliflower against *Plutella xylostella* was studied in pot culture experiments. The cauliflower plants were raised in pots to determine the persistence toxicity index (PTI) of the Spicturin against *P. xylostella.*

The plants were thoroughly sprayed with the following treatments using Ganesh sprayer® (1 litre capacity) and replicated four times. (1) Spicturin—2 ml/l (0.2%), (2) Spicturin—3 ml/l (0.3%), (3) Spicturin—4 ml/l (0.4%), (4) Control Replications: Four @ 10 larvae/replication.

The leaf samples were taken from the treated cauliflower plants one hour after spraying and brought to the laboratory for bioassay. The leaf samples were placed in plastic containers with moistened filter paper at the bottom to maintain the turgidity of leaves. Ten larvae pre-starved for 12 h were released in each replication. Adequate care was taken to provide enough treated leaf samples for first 24 h and thereafter fresh leaves were provided for the surviving larvae. Observations were made at 12 h intervals for the larval mortality and continued till the surviving insects pupated.

The leaf samples were taken from the treated plants at an interval of 24 h for conducting the similar bioassay and such bioassay studies were conducted till the mortality of larvae was reduced to zero.

The experiments were conducted for first and third instar larvae of *P. xylostella* in the first season (summer) in two sets of experiments. In one set of experiment, Teepol was added at the rate of 1 ml/l as a sticker along with Spicturin. In an another set, the product as such was used without addition of Teepol for spraying. Based on the results obtained from the above experiments, similar experiments were conducted with Spicturin and Teepol during winter season. In the case of control, Teepol @ 1 ml per litre of water was used for treating the plants. The Persistence Period (PP), Persistence Toxicity (PT) and PTI were worked out for the above experiments.

The PTI values for Spicturin @ 4, 3 and 2 ml/l were 1680, 1160 and 880 respectively for the first instar larvae (Table 1) and 1400, 960 and 720 respectively for the third instar larvae of *P. xylostella* on cauliflower (Table 2). The results showed that the efficacy of Spicturin with respect to the PTI values worked out was higher for the first instar (1680) when compare to the third instar (1400) larvae of *P. xylostella.* Similarly, Spicturin 3 and 2 ml/l recorded higher PTI values for the first instar larvae when compared to third instar larvae of *P. xylostella.*

TABLE 1. Persistent toxicity of Spicturin on cauliflower against first instar larvae of *P. xylostella* (summer season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	32.5	15.0	7.5	0	0	0	18.33	48	880	III
Spicturin 3 ml/l	37.5	22.5	12.5	0	0	0	24.17	48	1160	II
Spicturin 4 ml/l	50.0	37.5	17.5	0	0	0	35.00	48	1680	I

HAT - Hours after treatment. T - Average toxicity of the insecticides. P - Period of persistence. PTI - Persistent toxicity index.

TABLE 2. Persistent toxicity of Spicturin on cauliflower against third instar larvae of *P. xylostella* (summer season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	27.5	12.5	5.0	0	0	0	15.00	48	720	III
Spicturin 3 ml/l	30.0	20.0	10.0	0	0	0	20.00	48	960	II
Spicturin 4 ml/l	45.0	30.0	12.5	0	0	0	29.17	48	1400	I

TABLE 3. Persistent toxicity of Spicturin with Teepol (1 ml/l) on cauliflower against first instar larvae of *P. xylostella* (summer season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	65.0	37.5	22.5	12.5	0	0	34.38	72	2475	III
Spicturin 3 ml/l	72.5	45.0	27.5	15.0	0	0	40.00	72	2880	II
Spicturin 4 ml/l	80.0	45.0	37.5	20.0	10.0	0	38.50	96	3696	I

The PTI values for Spicturin @ 4, 3 and 2 ml/l along with Teepol were 3696, 2880 and 2475 respectively for the first instar larvae of *P. xylostella* on cauliflower (Table 3). The PTI values for Spicturin @ 4, 3 and 2 ml/l along with Teepol were 3456, 2475 and 1980 respectively for the third instar larvae of *P. xylostella* on cauliflower (Table 4). The efficacy of Spicturin @ 4 ml/l + Teepol with respect to the PTI values worked out was higher for the first instar (3696) when compared to third instar (3456) larvae of *P. xylostella* on cauliflower.

The PP of Spicturin with Teepol (1 ml/l) was found to be higher (ranging from 72 to 96 h) when compared to Spicturin alone (48 h) for the larvae of *P. xylostella*. Among the different doses tested, the order of PP was found to be higher with respect to Spicturin @ 4 ml/l with Teepol (1 ml/l) for the larvae of *P. xylostella* followed by Spicturin @ 3 ml + Teepol and Spicturin @ 2 ml/l + Teepol.

Irrespective of the doses and larval instars, Spicturin with Teepol was found to record higher persistence period when compared to Spicturin alone. The persistence toxicity of Spicturin @ 4 ml/l ranged from 17.5 to 7.5 per cent at 48 h as against

TABLE 4. Persistent toxicity of Spicturin with Teepol (1 ml/l) on cauliflower against third instar larvae of *P. xylostella* (summer season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	50.0	32.5	20.0	7.5	0	0	27.5	72	1980	III
Spicturin 3 ml/l	65.0	40.0	22.5	10.0	0	0	34.38	72	2475	II
Spicturin 4 ml/l	77.5	42.5	32.5	17.5	10.0	0	36.00	96	3456	I

TABLE 5. Persistent toxicity of Spicturin with Teepol (1 ml/l) on cauliflower against first instar larvae of *P xylostella* (winter season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	62.5	50.0	32.5	12.5	0	0	39.38	72	2835	III
Spicturin 3 ml/l	77.5	52.5	40.0	17.5	0	0	48.13	72	3465	II
Spicturin 4 ml/l	87.5	65.0	50.0	35.0	15.0	0	50.50	96	4848	I

10.0 per cent mortality at 96 h to Spicturin @ 4 ml + Teepol for the first instar larvae of *P. xylostella*.

The PTI values worked out for Spicturin @ 4, 3 and 2 ml/l were 4848, 3465 and 2835 respectively for the first instar larvae of *P. xylostella* on cauliflower (Table 5). The PTI values worked out for Spicturin @ 4, 3 and 2 ml/l were 4560, 3195 and 2565 respectively for the third instar larvae of *P. xylostella* on cauliflower (Table 6).

The PP of Spicturin @ 4 ml/l + Teepol was found to be 96 h as against 72 h in Spicturin @ 3 and 2 ml/l for first and third instar larvae of *P. xylostella*.

The results indicated that the PTI values of Spicturin showed a marked variation between different doses of Spicturin irrespective of different instars of *P. xylostella*. Similarly, the PTI, PT and PP of Spicturin on the host plant, cauliflower against *P. xylostella* was increased as the doses increased and were higher in Spicturin @ 4 ml/l followed by Spicturin @ 3 and 2 ml/l. The present investigations are in support to the findings of Tata Rao (1998) who reported that the persistent toxicity of Spicturin was greater at the dose of 4 ml/l followed by Spicturin @ 3 and 2 ml/l on chickpea *against H. armigera* and on groundnut against *S. litura*. The PT and PTI of Spicturin was higher against the first instar when compared to the third instar irrespective of the doses of Spicturin and PT was declined as the larval instars progressed as reported by Tata Rao (1998).

In the present investigation, the PP and PT were higher for Spicturin applied along with Teepol when compared to Spicturin applied alone irrespective of the doses of Spicturin and larval instars of *P. xylostella*. It is quite evident that the surfactant, Teepol played a major role in enhancing the persistence of *B.t.* on the host plants. Earlier reports also indicated differences because of the variations in the physical characteristics of the formulations (Pinnock *et al.*, 1971). Solar radiation might be

TABLE 6. Persistent toxicity of Spicturin with Teepol (1 ml/l) on cauliflower against third instar larvae of *P. xylostella* (winter season)

Treatment	Mortality (%) HAT						T	P	PTI	Rank
	1	24	48	72	96	120				
Spicturin 2 ml/l	57.5	42.5	30.0	12.5	0	0	35.63	72	2565	III
Spicturin 3 ml/l	65.0	52.5	40.0	15.0	0	0	44.38	72	3195	II
Spicturin 4 ml/l	77.5	60.0	52.5	32.5	15.0	0	54.50	96	4560	I

a major factor affecting the persistence of *B.t.* on treated leaves as opined by Pinnock *et al.* (1974) and also adverse effect of sunlight, U.V. rays and other environmental factors like rain wash-off, plant phenolics etc. (Patel and Vyas, 1997).

However, Spicturin @ 4 ml/l along with Teepol persisted for 96 h on cauliflower against *P. xylostella* during both the seasons. In contrary to this, eight days of persistence of Thuricide 90TS was reported by Verma and Gill (1977) and of Spicturin (Justin, 1996) on cauliflower. Similarly Biotrol persisted on cabbage for 10 days (Rajamohan and Jayaraj, 1978). However, Mohamad *et al.* (1980) observed 50 per cent mortality of second instar larvae of *P. xylostella* six days after *B.t.* spray on turnip. Bertona *et al.* (1994) also reported that the biopesticide, Delfin was effective for 7–10 days after application and its degradation was mainly due to light and temperature. Salama *et al.* (1983) reported the persistence of *B.t.* spores on cotton elucidating an obvious reduction one day after spraying and the delay in spore viability was progressively correlated with the time of exposure in the field and the half-life of the tested spores of *B.t.* formulations varied between 75 and 256 h. Within the insect, the decomposition of the crystal was dependent on the species of insect, the developmental stage of insect and the food source of the insect. The variations in the persistent toxicity of *B.t.* to various instars of insect might be due to the rate of food consumption and the food particle size suitable for digestion.

From the present investigations, it is concluded that indigenous *B.t.* formulation, Spicturin can be used at the higher dose of 4 ml/l along with the surfactant, Teepol @ 1 ml/l for the management of *P. xylostella* on cauliflower and it will persist on plant for longer time to cause disease to the larvae of *P. xylostella*.

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REFERENCES

Beegle, C. C. and Yamamoto, T. (1992) Invitation paper (C. P. Alexander Fund): History of *bacillus thuringiensis* Berliner research and development, *Can. Ent.* **124**: 587–616.

Bertona, A., Compagna, C. and Pasoquali, F (1994) Delfin: a new preparation based on *Bacillus thuringiensis*, *Information Fitopatologico* **44(7–8)**: 20–26.

Gujar, G. T., Archanakumari, and Vinay Kalia, (1998) Bioefficacy of *Bacillus thuringiensis* subspecies *kurstaki* strains and formulations against *Helicoverpa armigera, ICPPMSA, (11–13 Dec. 1998)*, CSAU A&T: Kanpur, 148 p.

Justin, C. G. L. (1996) *Bacillus thuringiensis* Berliner in the IPM of Diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on cauliflower *Ph.D. Thesis*, Tamil Nadu Agricultural University, Coimbatore, India, 187 p.

Knowles, B. H. (1994) Mechanism of action of *Bacillus thuringiensis* insecticidal delta-endotoxin, *Adv. Insect Physiol.* **24**: 275–308.

Mohamad, R., Ibrahim, Y. and Chong, W. C. (1980) The residual effect of some insecticides on *Plutella xylostella* (L.) larvae in the greenhouse, *Pertanika* **3(1)**: 10–12.

Patel, M. C. and Vyas, R. V. (1997) Persistence of *Bacillus thuringiensis* var. *kurstaki* formulations on cotton hybrid, In: *Proc. National Seminar on Plant Protection Towards Sustainability (Dec. 22–24, 1997)*, NPPTI: Hyderabad. *Indian J. Plant Prot.* **25(2)**: 165–174.

Pinnock, D. E., Brand, R. J. and Milstead, J. E. (1971) The field persistence of *Bacillus thuringiensis* spores, *J. Invertebr. Pathol.* **18**: 405–411.

Pinnock, D. E., Brand, R. J., Jackson, K. L. and Milstead, J. E. (1974) The field persistence of *Bacillus thuringiensis* spores on *Cercis occidentalis* leaves, *J. Biol.* **23**: 341–346.

Rajamohan, N. and Jayaraj, S. (1978) Field efficacy of *Bacillus thuringiensis* and some other insecticides against pests of cabbage, *Indian J. Agric. Sci.* **48**: 672–675.

Salama, H. S., Foda, M. S., Zaki, F. N. and Khalafallah, A. (1983) Persistence of *Bacillus thuringiensis* Berliner spores in cotton cultivars, *Z. Dug. Ent.* **95**: 321–326.

Tata Rao, K. S. R. (1998) Efficacy of spicturin on certain crop pests *M.Sc. (Ag.) Thesis*, Tamil Nadu Agricultural University Coimbatore, India, 98 p.

Verma, G. C. and Gill, G. S. (1977) Laboratory studies in the comparative efficacy of biotic insecticides for the control of *Plutella xylostella, J. Res., Punjab Agric. Univ.* **14**: 304–308.

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Relative resistance in teak clones to leaf skeletonizer, *Eutectona machoeralis* (Walker) (Lepidoptera: Pyralidae) and role of leaf moisture

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ABSTRACT: Fourteen clones of *Tectona grandis* L.f. belonging to five states of India (Andhra Pradesh, Tamilnadu, Orissa, Maharashtra, and Uttar Pradesh) were screened for relative resistance against the insect pest, *Eutectona machoeralis* (Walker) (Lepidoptera: Pyralidae). On the basis of leaf area consumed/day/larva of fourth and fifth instar, resistance in clones were arranged in descending order of magnitude: APT-20, MHAL-A5, APNPL-7, MHAL-A2, APKEA-24, TNT-10, ORANP-5, APT-14, TNT-3, UP-C, MHAL-A3, MHAL-A4, APMN-4 and MHAL-A1. Water content of contributory leaves of teak clones revealed a gradual increase in relation to leaf area consumption. The results are discussed in the light of the present findings.

KEYWORDS: Resistance, teak, *Eutectona machoeralis*, leaf moisture

After realization of immense importance of host resistance in forest trees (Hanover, 1980), clonal resistance in teak (*Tectona grandis* L.f.) provides a highly practical approach to manage insect problems (Kedharnath, 1984; Tewari, 1992; Nair *et al.*, 1997). Concerted efforts for utilizing insect resistance in teak improvement programme started with the preliminary observations of Kedharnath and Singh (1975) on some teak clones against leaf skeletonizer, *Eutectona machoeralis* (Walker) (Lepidoptera: Pyralidae), a major insect pest of economic importance. Ahamad (1991), Misra (1992), Meshram (1993) and Meshram *et al.* (1994) have carried out studies on the same insect-host interactions. Recently, an exploratory research programme has been carried out in this direction to evaluate resistant teak clones against *E. machoeralis* and to ascertain the factors responsible for natural resistance in teak (Roychoudhury and Joshi, 1996). The present work is a part of such an exploratory programme to find out natural relative resistance of certain clones of teak to skeletonizer, based on feeding trails in laboratory, and role of leaf moisture on feeding potentiality of this pest.

Larvae of *E. machoeralis* were collected from the heavily infested teak trees as mixed populations of different larval instars. Freshly ecdysed, fourth and fifth instar

TABLE 1. Leaf area consumption of *E. machoeralis* on different teak clones and water content of contributory leaves

Clone	Source/origin	Leaf area consumed (sq. cm) in 24 hours		Moisture (%)
		Instar IV	Instar V	
APT-20	Andhra Pradesh	0.80	1.96	60.27 (50.93)
MHAL-A5	Maharashtra	1.50	2.04	60.96 (51.34)
APNPL-7	Andhra Pradesh	1.52	2.08	62.61 (52.35)
MHAL-A2	Maharashtra	1.70	2.24	63.00 (52.52)
APKEA-24	Andhra Pradesh	1.74	2.42	66.11 (54.44)
TNT-10	Tamilnadu	1.80	2.56	69.07 (56.24)
ORANP-5	Orissa	2.04	2.58	69.71 (56.61)
APT-14	Andhra Pradesh	2.18	2.64	69.91 (56.73)
TNT-3	Tamilnadu	2.18	2.66	71.72 (57.92)
UP-C	Uttar Pradesh	2.20	3.10	71.95 (58.02)
MHAL-A3	Maharashtra	2.48	3.14	72.02 (58.07)
MHAL-A4	Maharashtra	2.54	3.66	73.18 (58.81)
APMN-4	Andhra Pradesh	3.34	4.04	73.31 (59.89)
MHAL-A1	Maharashtra	3.42	6.30	73.92 (59.30)
SEM		0.489	0.539	0.923
CD at 1%		1.311	1.446	2.565
CD at 5%		0.982	1.083	1.898

Angular transformed values are inside parentheses.

larvae were separated out (Roychoudhury and Joshi, 1993), then starved for about 6 hours and were used in the present study.

To investigate relative resistance in teak clones to *E. machoeralis*, 14 selected clones of teak belonging to five states of India (Andhra Pradesh, Tamilnadu, Orissa, Maharashtra and Uttar Pradesh) were considered as experimental host plants, based on earlier work (Roychoudhury and Joshi, 1996). Grafts of these clones were collected from National Teak Germ Plasm Bank, Lohara, Chandrapur (Maharashtra) and were maintained in polybag at Tropical Forest Research Institute (Madhya Pradesh). Counting from the tip of a twig, 3rd pair of leaves of these 10 months old clones was used in the present work. Freshly ecdysed fourth (0.005–0.009 g) and fifth (0.020–0.25 g) instar larvae (Roychoudhury and Joshi, 1993) were allowed to feed for 24 hours on measured (13 sq. cm) equal sized leaf discs (1 larva/disc) of different clones separately. Then, leaf discs were removed, leaf area was measured individually with the help of leaf area meter (Systronics 211). Some of the contributory leaves of different clones were weighed separately, then dried to constant weight at 60 °C for 48 hours to find out the water contents of leaves (Roychoudhury *et al.*, 1995). The data ($n = 10$) on leaf area consumed and moisture percentage of leaves were subjected to statistical analysis by ANOVA (CRD) test. The experiment was conducted under the prevailing environmental conditions of October, 1998 (temp. 20–33 °C and RH 46–63%).

Data on mean leaf area consumed by the fourth and fifth instar larvae of *E. machoeralis* on different teak clones are arranged in descending order of magnitude (Table 1) and considered as a basis for evaluation of natural relative resistance among the clones. Results revealed significant ($P < 0.05$–$P < 0.01$) variation in their mean values in regard to leaf area consumption of teak clones by fourth and fifth instar larvae of teak leaf skeletonizer. Among the clones tested, clone APT-20 of Andhra Pradesh was least preferred whereas clone MHAL-A1 of Maharashtra was noticed to be the highly favourable host, both the fourth and fifth instar larvae. The intra-state comparison of teak clones also exhibited significant ($P<0.05$–$P<0.01$) variations in leaf area consumption against fourth and fifth larval instars. All these findings strongly suggest the existence of resistance phenomenon in teak clones which conform the field observations of Roychoudhury and Joshi (1996) on 167 clones of teak, at National Germ Plasm Bank, Lohara, Chandrapur (Maharashtra). The results of the present work corroborate the finding of previous workers (Ahamad, 1991; Misra, 1992) on some other teak clones against *E. machoeralis*. Earlier, Meshram (1993) and Meshram *et al.* (1994) have investigated on the basis of larval leaf area consumption that clone APT-20 is least preferred to teak leaf skeletonizer. The same result has also been emerged out from the present study.

The water contents of contributory leaves of different teak clones revealed a gradual increase of moisture percentage in respect to leaf area consumption (Table 1) and showed significant ($P<0.05$–$P<0.01$) difference in their mean values. Least preferred clone, APT-20 exhibited lowest leaf moisture whereas most susceptible clone MHAL-A1 showed maximum percentage of leaf moisture. The results clearly indicate that water contents of leaves of teak clones may have a role in alteration of feeding potentiality of *E. machoeralis*, for which larvae exhibit feeding preference among the clones. This is possible because leaf water, frequently overlooked as a nutrient, can also have a major influence on insect performance (Scriber and Slansky, 1981). High leaf moisture is associated with an increased quantity of food consumed while a low water content affects energy expenditure and nutritional efficiency of insects (Matson and Scriber, 1987).

The water uptake in plant and holding capacity in leaves differ according to plant species and variety (Slansky and Scriber, 1985; Bose *et al.*, 1991). The present study clearly indicates that water holding capacity in leaves of teak clones varies. Based on leaf area consumption by the larvae of *E. machoeralis*, least preferred teak clones possess lower moisture content whereas high moisture percentage exists in most preferred teak clones.

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REFERENCES

Ahmad, M. (1991) Natural resistance in teak clones to leaf skeletonizer, *Eutectona machaeralis* (Lepidoptera: Pyralidae), *Indian J. Forestry* **14(3)**: 228–231.

Bose, P. C., Majumder, S. K. and Sengupta, K. (1991) A comparative biochemical study of six mulberry (*Morus alba* L.) varieties, *Indian J. Seric.* **30(1)**: 83–87.

Hanover, J. W. (1980) Breeding forest trees resistant to insects, In: *Breeding Plants Resistant to Insects*, Maxwell, F. G. and Jennings, P. R. (Editors). John Wiley & Sons: New York, 487–511.

Kedharnath, S. (1984) Forest tree improvement in India, *Proc. Indian Acad. Sci. (Plant Sci.)* **93(3)**: 401–412.

Kedharnath, S. and Singh, P. (1975) Studies on natural variation in susceptibility of *Tectona* to leaf skeletonizer, *Pyrausta machaeralis* (Lepidoptera: Pyralidae), In: *FAO/IUFRO Symposium on Forest Diseases & Insects (7–12 April) New Delhi, India.*

Matson, W. J. and Scriber, J. M. (1987) Nutritional ecology of insect folivores of woody plants: nitrogen, water, fibre and mineral considerations, In: *Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrate*, Slansky, F. Jr. and Rodriguez, J. G. (Editors). John Wiley & Sons: New York, 105–146.

Meshram, P. B. (1993) Relative resistance of certain clones of *Tectona grandis* to *Eutectona machaeralis* Walk, *J. Applied. Zool. Res.* **4(1)**: 87–88.

Meshram, P. B., Joshi, K. C. and Sarker, A. K. (1994) Relative resistance of certain clones of *Tectona grandis* to teak leaf skeletonizer, *Eutectona machaeralis* Walk. (Lepidoptera: Pyralidae), *Indian Forester* **120(1)**: 58–61.

Misra, S. C. (1992) Comparative natural resistance of different clones of *Tectona grandis* L.f. to teak skeletonizer, *Eutectona machaeralis* Walk. (Lepidoptera: Pyralidae), *Indian Forester* **118(4)**: 274–278.

Nair, K. S. S., Sudheendrakumar, V. V., Mohanadas, K., Varma, R. V., Mathew, G., Koshy, M. P. and Kedharnath, S. (1997) Search for teak trees resistant to the defoliator, *Hyblaea puera* Cramer (Lepidoptera, Hyblaeidae), In: *Ecology and Evolution of Plant-Feeding Insects in Natural and Man-Made Environments*, Raman, A. (Editor). International Scientific Publications: New Delhi, 109–122.

Roychoudhury, N. and Joshi, K. C. (1993) Growth and developmental fate of moisture in teak leaf skeletonizer, *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae), *J. Trop. Biod.* **1(3&4)**: 296–301.

Roychoudhury, N. and Joshi, K. C. (1996) Search for natural resistance in teak clones against *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae), *Indian J. Forestry* **19(3)**: 205–213.

Roychoudhury, N., Jain, A. and Joshi, K. C. (1995) Alteration of growth and development in leaf skeletonizer, *Eutectona machaeralis* Walker, due to variations in teak leaves of different maturity, *Indian J. Exp. Bio.* **33(3)**: 227–229.

Scriber, J. M. and Slansky, F. Jr. (d1981) Nutritional ecology of immature insects, *Annu. Rev. Entomol.* **26**: 83–211.

Slansky, F. Jr. and Scriber, J. M. (1985) Food consumption and utilization, In: *Comprehensive Insect Physiology Biochemistry and Pharmacology*, Vol. 4. Kerkut, G. I. and Gilbert, L. I. (Editors). Pergamon Press: Oxford, 88–163.

Tewari, D. N. (1992) *A Monograph on Teak (Tectona grandis Linn. f)*, International Book Distributors: Dehradun, India, 479 p.

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Effect of starvation and density on the nymphal cannibalism of *Rhynocoris fuscipes* Fab. (Heteroptera: Reduviidae)

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ABSTRACT: Different nymphal stage of *Rhynocoris fuscipes* (I, II, II, IV and V) nymphal instars) were reared in groups in two different sizes of containers and also starved for 2, 4 and 6 days respectively and the cannibalistic behaviour in each condition was noted. Remarkable cannibalism was noted in all the observations. However it was enhanced by density. Similarly the rate of starvation also had marked influence on the cannibalistic activity. Among the nymphal instars cannibalism was maximum in the first nymphal instar followed by second nymphal instar. Lowest cannibalism was observed in the third nymphal instar.

KEYWORDS: *Rhynocoris fuscipes*, nymphs, cannibalism, space, starvation

INTRODUCTION

Rhynocoris fuscipes Fab. is a polyphagous reduviid predator found to be predating on various insect pests both in the laboratory and in the field (Ambrose, 1999). Nymphal cannibalism is a major constraint in the mass multiplication of this reduviid in the laboratory. In order to have a sound knowledge on the nymphal cannibalism of various nymphal instars, an attempt has been made to study the effect of starvation and insect density on the cannibalistic behaviour of this reduviid.

Adults and nymphs of *R. fuscipes* were collected from Kannankulam cotton fields, Tirunelveli district, Tamil Nadu, South India (latitude 77° 32.31 E and 8° 10.38 N). They were maintained in the laboratory in plastic containers at 30 ± 2° C, RH ranging from 75–80% and photoperiod between 11 and 13 h on the larvae of *Spodoptera litura* Fabricius. Freshly moulted nymphs were used for the present experiment to study the cannibalistic behaviour.

Two hundred first instar nymphs of *R. fuscipes* were recruited from the stock culture and divided into two groups to study the effect of mass rearing, density and prey deprivation on cannibalism. Twenty five nymphs each, were kept in four large plastic troughs (less crowded) (16 × 10 cm). Simultaneously, twenty five nymphs each, were placed in four small plastic troughs (much crowded) (10 × 7 cm). The four troughs in each category were further experimented into 4 different conditions *viz.*, daily fed and prey deprived for 2, 4 and 6 days respectively. The rates of cannibalism in all the eight categories were recorded (as complete (C) or partial (P) cannibalism). The experiment was repeated with II, III, IV and V nymphal instars separately.

A higher percentage of cannibalism was invariably registered among the first nymphal instar both for fed or starved but it was considerably lower for the succeeding instars. This was seen both in less crowded and more crowded conditions. Highest cannibalism in first nymphal instar was followed by II nymphal instar whereas in the III nymphal instar the extent of cannibalism was the least. Slightly increased cannibalism was noted in the IV and V nymphal instars. This was in agreement with the earlier findings of Ambrose (1986).

Out of 25 first instar nymphs reared under less crowded condition (in large trough) 5 insects were cannibalised (2 insects cannibalised completely and 3 partially) in daily fed condition and this figure increased to 9, 13 and 17 insects when starved for 2, 4 and 6 days, respectively. The percentage of cannibalism increased from 20 in daily fed nymphs to 36, 52 and 68 in 2, 4 and 6 days starved nymphs, respectively. Similar increased percentage of cannibalism was recorded for the starved II, III, IV and V nymphal instars (Table 1). From these results it appeared that starvation accelerated cannibalism as noted by Iqbal and Aziz (1976) and Sofi and Bhat (1997) in a grasshopper *Spathosternum* and Cloarec (1985) in water stick insect. Daily feeding in a prerequisite for the laboratory rearing of *R. marginatus*. Ambrose *et al.* (1985a; 1985b; 1990; 1992), Ambrose and Amudha (1987), Ambrose and Sahayaraj (1990) and Ambrose and Claver (1996) noted that starvation accelerated the predatory behaviour and delayed the post embryonic development, in some reduviids.

The cannibalistic rate was found to increase as a function of insect density. In the insects reared under much crowded condition, the rate of cannibalism was more than those reared under less crowded condition. For instance, in first instar nymphs reared under much crowded condition the percentage of cannibalism increased from 20 to 28, 36 to 52, 52 to 72 and 68 to 92% respectively in 0, 2, 4 and 6 days starved individuals. Similar increased rate of cannibalism was also observed for all the other four nymphal instars. Similar observation were made by Iqbal and Aziz (1976) and Sofi and Bhat (1997).

The present study in the laboratory gives conducive conditions regarding the space and food to conserve and augment this potential biological control agent for mass multiplication.

TABLE 1. Effect of prey deprivation and density on the cannibalistic behaviour of *R. fuscipes*

Size of the predators	Size of the trough	Number of insects cannibalised after starvation								Percentage of insects cannibalised after starvation			
		0 day		2 days		4 days		6 days		0 day	2 days	4 days	6 days
		C	P	C	P	C	P	C	P				
I instar	Large	2	3	4	5	6	7	7	10	20	36	52	68
	Small	3	4	6	7	8	10	10	13	28	52	72	92
II instar	Large	1	2	4	4	4	6	5	9	12	32	40	56
	Small	2	3	5	6	5	8	8	11	20	44	52	76
II instar	Large	0	2	2	2	2	3	3	5	8	16	20	32
	Small	1	2	2	4	3	5	4	7	12	24	32	44
IV instar	Large	0	3	3	3	3	4	4	6	12	24	28	40
	Small	2	3	3	5	3	6	6	8	20	32	36	56
V instar	Large	2	2	3	4	4	5	5	6	16	28	36	44
	Small	2	3	4	6	5	6	6	10	20	40	64	

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REFERENCES

Ambrose, D. P. (1986) Impact of nymphal camouflaging on predation and cannibalism in the bug, *Acanthaspis siva. Environment & Ecology* **4(2)**: 197–200.

Ambrose, D. P. (1999) *Assassin bugs*, Oxford and IBH Publishing Co. Ltd.: New Delhi, 262–284.

Ambrose, D. P. and Amudha, R. C. (1987) Impact of starvation and competition in the preying behaviour of *Acanthaspis pedestris* Stal. (Insecta: Heteroptera: Reduviidae), *Research Review* **4(1)**: 1–12.

Ambrose, D. P. and Sahayaraj, K. (1990) Effect of space on the post embryonic development and predatory behaviour of *Ectomocoris tibialis* Distant (Insecta: Heteroptera: Reduviidae), *Uttar Pradesh J. Zool.* **10(2)**: 163–170.

Ambrose, D. P. and Claver, M. A. (1996) Impact of prey deprivation in the predatory behaviour of *Rhynocoris kumarii* Ambrose and Livingstone (Heteroptera: Reduviidae), *J. Soil Biol. & Ecol.* **16(1)**: 78–87.

Ambrose, D. P., Solomon, D. and Vennison, S. J. (1985a) Effect of competition, space and starvation on the predatory behaviour of the bug *Rhynocoris marginatus*, *Environment & Ecology* **3(2)**: 280–285.

Ambrose, D. P., Lazar, X. and Jebanesan, A. (1985b) Effect of starvation and competition on the predatory behaviour of *Catamiarus brevipennis* Serville (Insecta: Heteroptera: Reduviidae), *Bull. Environ. Sci.* **2(2)**: 1–6.

Ambrose, D. P., Sekar, P. C. and Kumaraswami, N. S. (1990) Effect of starvation on the development, reproduction and size of assassin bug *Rhynocoris marginatus*, *Environment & Ecology* **8(2)**: 548–555.

Ambrose, D. P., Gomathivinayagam, P. and Rajan, K. (1992) Impact of starvation on the post embryonic development and bioenergetics of *Acanthaspis pedestris* Stal. (Insecta: Heteroptera: Reduviidae), *Environment & Ecology* **10(3)**: 675–681.

Cloarec, A. (1985) Persistence of deprivation effects in the water stick insect, *Biol. & Behavi.* **10**: 279–289.

Iqbal, M. and Aziz, S. A. (1976) Cannibalism in *Spathosternum parasinifernum* Walk, *Ind. J. Zool.* **4(2)**: 43–45.

Sofi, A. M. and Bhat, M. I. (1997) Cannibalistic behaviour of adults of grasshopper *Spathosternum parasinifernum* in relation to different conditions of feeding, temperature and density, *Ind. J. Ent.* **59(2)**: 141–146.

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Efficacy of *Bacillus thuringiensis* var. galleriae (Spicturin) against *Helicoverpa armigera* on chickpea

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ABSTRACT: Field experiments were conducted at Kurumbapalayam, Coimbatore district, during 1997–98 to test the efficacy of *Bacillus thuringiensis* var galleriae (Spicturin) at different concentrations and compared with cartap on the *Helicoverpa armigera* larval population, pod damage and yield of chickpea. Significant reduction in larval population of *H. armigera* was recorded in spicturin @ 2 lit/ha followed by cartap 50% SP 2.5 kg/ha. The pod damage was the lowest in spicturin 2.00 lit/ha followed by cartap 50% SP 2.5 kg/ha. The maximum yield of chickpea was obtained from spicturin treatment.

KEYWORDS: *Bio-efficacy, Bacillus thuringiensis (B.t.g.), Helicoverpa armigera*, chickpea

INTRODUCTION

The gram caterpillar, *Helicoverpa armigera* (Hub.) is a dominant and serious pest of chickpea in India (Pimpert, 1990). *H. armigera* infestation on chickpea is very severe and causes pod damage upto 90 per cent (Shengal and Ujagir, 1990). In Tamil Nadu, the loss caused by *H. armigera* to chickpea was estimated as 40 per cent during 1987–88 (Jayaraj, 1990).

Indiscriminate use of pesticides has resulted in development of resistance to cypermethrin, endosulfan and fenvalerate by *H. armigera* (Dhingra *et al.*, 1988). The recommended conventional broad spectrum insecticides also do not provide satisfactory control of the pest. These situations warranted the development of alternate method of pest management.

One such envisaged method is the use of microbes, particularly *Bacillus thuringiensis*. Berliner which is of growing importance in recent years and it is less persistent, specific and safer. *B.t.* is the most important microbial agent emerged as a competitive bio-insecticide after extensive field trials and safety testing (Burgus, 1981). Based

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on the above characteristics, the efficacy of *B. thuringiensis* var galleriae (spicturin) developed by Tuticorin Alkali Chemicals and Fertilizers Ltd, Chennai was evaluated against gram caterpillar, *H. armigera* on chickpea and the results are presented in this paper.

In order to evaluate the efficacy of 'spicturin' (*B.t.g*) against *H. armigera* on chickpea, two field experiments were conducted at Kurumbapalayam area of Coimbatore district during 1997–98. The trials were laid out in a RBD design with five treatments, replicated four times with the plot size of 20 m^2 per replication. The treatments consisted of *B.t.g* @ 1.0, 1.5, 2.0 lit/ha, an animal toxin from *Nerris lumbiricoidus* (cartap) 50% SP 2.50 kg/ha and an untreated check. The surfactant, Teepol was added @ 1 ml per litre of water to the treatments. Five rounds of sprays were given using the hand operated Knapsac sprayer when the population of *H. armigera* exceeded the ETL of one larva/plant in any one replication. The number of larvae, the total number of pods and damaged pods in each plot/replication were recorded on ten plants selected at random for the above observations. The observations were made at three stages *viz.*, pre treatment, third and seventh day after each spraying. The pod damage and the pod yield per replication were recorded at harvest. The per cent damage due to the pod borer was worked out and yield data were computed to hectare.

In the first experiment, the pre-treatment population ranged from 2.30 to 3.13 larvae/plant. The final population ranged from 0.05/plant in *B.t.g* @ 2 lit/ha which was significantly superior to other treatments. The next in order was cartap 2.5 lit/ha with a population of 0.15/plant. The per cent reduction in larval population over control was maximum in *B.t.g.* 2 lit/ha (97.50) followed by cartap 2.5 kg/ha (92.50). Pod damage was lower in *B.t.g.* (11.49%) and cartap (12.55%) while in untreated check it was 71.5 per cent.

All the biocidal treatments were significantly superior in recording higher yield when compared to untreated check. Maximum yield was recorded in *B.t.g.* 2 lit/ha (2,600 kg/ha) followed by 2,410 kg/ha in cartap (2.5 kg/ha) while the lowest yield of 1,350 kg/ha was obtained in untreated plots (Table 1).

The pre-treatment population ranged from 2.00 to 3.40 per cent in the second experiment. A minimum population of 0.08 larva per plant was noticed in *B.t.g.* 2 lit/ha indicating its superior efficacy in reducing the larval population. The next in order in recording less larval population were with cartap 2.5 kg/ha (0.2/plant) and spicturin 1.5 lit/ha (0.28/plant) which were on par. The final reduction in population over control was maximum (96.44%) in *B.t.g.* 2 lit/ha followed by 91.03 per cent in cartap 2.5 kg/ha. The damage was the lowest in *B.t.g.* 2 lit/ha (12.77%) followed by cartap 2.5 kg/ha (13.56%) which were on par.

Maximum grain yield was obtained in *B.t.g.* 2 lit/ha (2,575 kg/ha) followed by 2,410 kg/ha in *B.t.g.* 1.5 lit/ha (Table 2).

The significant effectiveness of *B.t.g.* @ 2 lit/ha and cartap @ 2.5 lit/ha in reducing the larval population of *H. armigera* and realising higher grain yield in chickpea is in agreement with the results of Peter *et al.* (1989) who found out the superior efficacy of cartap in controlling the larvae of diamond back moth (DBM), *Plutella xylostella*

TABLE 1. Efficacy of *Bacillus thuringiensis* var. *galleriae* (Spicturin) in chickpea (Trial I)

Sl. No.	Treatments	Pre-treatment	Number of larvae/plant* @ (DAS - Days after spraying)										Reduction (%)	Pod damage (%) over control	Yield kg/ha
			I Spray		II Spray		III Spray		IV Spray		V Spray				
			3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS			
1	Spicturin 1.0 lit/ha	3.25	1.38 (1.37)^{a}	1.53 (1.42)^{c}	2.40 (1.69)^{b}	1.60 (1.45)^{c}	1.68 (1.47)^{b}	1.90 (1.55)^{c}	1.85 (1.50)^{b}	1.63 (1.46)^{d}	1.33 (1.35)^{c}	1.18 (1.47)^{c}	41.00	37.49 (37.74)^{c}	1875
2	Spicturin 1.5 lit/ha	3.08	1.18 (1.29)^{a}	0.98 (1.21)^{b}	1.90 (1.55)^{a}	1.35 (1.36)^{b}	1.23 (1.37)^{a}	1.08 (1.26)^{b}	1.08 (1.26)^{a}	0.70 (1.09)^{c}	0.83 (1.15)^{b}	0.23 (1.10)^{b}	88.50	24.76 (29.83)^{b}	2185
3	Spicturin 2.0 lit/ha	2.73	1.18 (1.29)^{a}	0.53 (1.01)^{a}	1.60 (1.45)^{a}	1.18 (1.29)^{a}	1.00 (1.23)^{a}	0.73 (1.11)^{a}	0.65 (1.07)^{a}	0.35 (0.92)^{a}	0.33 (1.91)^{a}	0.05 (1.03)^{a}	97.50	11.49 (19.79)^{a}	2600
4	Cartap 50 % SP 2.5 kg/ha	2.30	1.33 (1.43)^{a}	1.63 (1.44)^{c}	1.80 (1.52)^{a}	1.25 (1.32)^{ab}	1.08 (1.26)^{a}	1.05 (1.25)^{b}	0.68 (1.08)^{a}	0.55 (1.02)^{a}	0.40 (1.95)^{a}	0.15 (1.07)^{b}	92.50	12.55 (20.74)^{a}	2410
5	Control	3.13	1.48 (1.40)^{b}	1.98 (1.56)^{c}	4.63 (2.26)^{b}	4.10 (2.28)^{d}	2.65 (1.77)^{c}	3.00 (1.87)^{d}	2.58 (1.75)^{c}	2.48 (1.72)^{c}	2.43 (1.71)^{d}	2.00 (1.73)^{d}	—	71.51 (57.76)^{d}	1350

* Mean of four replications. Means followed by same letter do not differ dignificantly by DMRT ($P = 0.05$). Figures in parentheses are square root transformed values.

TABLE 2. Efficacy of *Bacillus thuringiensis* var. *galleriae* (Spicturin) in chickpea (Trial II)

Sl. No.	Treatments	Pre-treatment	Number of larvae/plant* @ (DAS - Days after spraying)										Reduction (%)	Pod damage (%) over control	Yield kg/ha
			I Spray		II Spray		III Spray		IV Spray		V Spray				
			3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS	3 DAS	7 DAS			
1	Spicturin 1.0 lit/ha	2.80	1.30 (1.34)^{bc}	1.35 (1.36)^{c}	2.43 (1.71)^{a}	1.67 (1.47)^{c}	1.78 (1.51)^{c}	1.85 (1.53)^{d}	1.70 (1.48)^{c}	1.60 (1.45)^{d}	1.25 (1.32)^{c}	1.18 (1.47)^{c}	47.10	39.01 (38.64)^{c}	1750
2	Spicturin 1.5 lit/ha	2.38	1.28 (1.33)^{b}	1.33 (1.35)^{c}	2.00 (1.57)^{a}	1.43 (1.39)^{b}	1.20 (1.31)^{b}	1.08 (1.26)^{b}	0.93 (1.20)^{b}	0.80 (1.14)^{c}	0.65 (1.07)^{b}	0.28 (1.13)^{b}	87.44	21.59 (27.71)^{b}	2410
3	Spicturin 2.0 lit/ha	2.00	1.23 (1.32)^{b}	0.43 (0.96)^{a}	1.68 (1.47)^{a}	1.08 (1.26)^{a}	0.95 (1.21)^{a}	0.78 (1.13)^{a}	0.70 (1.12)^{a}	0.43 (0.96)^{a}	0.20 (0.57)^{a}	0.08 (1.04)^{a}	96.41	12.77 (20.92)^{a}	2575
4	Cartap 50 % SP2.5 kg/ha	2.83	0.90 (1.19)^{a}	1.10 (1.26)^{b}	1.88 (1.54)^{a}	1.15 (1.29)^{a}	1.00 (1.23)^{a}	0.83 (1.17)^{b}	0.63 (1.06)^{a}	0.65 (1.07)^{b}	0.38 (0.85)^{a}	0.20 (1.21)^{b}	91.03	13.56 (21.26)^{a}	2365
5	Control	3.40	1.45 (1.39)^{c}	1.60 (1.45)^{c}	4.15 (2.16)^{b}	4.47 (2.23)^{d}	2.88 (1.84)^{d}	2.35 (1.69)^{d}	2.43 (1.70)^{d}	2.43 (1.71)^{e}	2.43 (1.71)^{d}	2.33 (1.79)^{d}	—	71.69 (58.89)^{d}	1150

* Mean of four replications. Means followed by same letter do not differ dignificantly by DMRT ($P = 0.05$). Figures in parentheses are square root transformed values.

larvae on cabbage while Nagesh and Shashiverma (1997) who reported that, cartap at 0.05 per cent concentration was found to be effective in controlling the diamond back moth (DBM), *Plutella xylostella* followed by *B.t.* (Biolep, 0.2%) and also recording higher yield of cabbage heads.

Hence, it can be concluded that the eco-friendly insecticides of spicturin 2 lit/ha followed by cartap 2.5 kg/ha may be recomended for the control of *H. armigera* larvae on chickpea.

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REFERENCES

Burgus, K. D. (1981) *Microbial Control of Pests and Plant Diseases 1970–80*, Academic Press: London, 737–767.

Dhingra, S., Phokela, A. and Mehrotra, K. W. (1988) Cypermethrin resistant in the population of *Helicoverpa armigera* (Hub.), *Nat. Acad. Sci.* (eff.) **11**: 23–125.

Jayaraj, S. (1990) The problems of *Heliothis armigera* in India and its Integrated Management, In: *Heliothis Management: Proc. Natl. Workshop*, Jayaraj, S., Uthamaswamy, S., Goplan, M. and Rabindra, R. J. (Editors). Tamil Nadu Agricultural University: Coimbatore, 1–16.

Nagesh, M. and Shashiverma, (1997) Bioefficacy of certain insecticides against diamond back month (*Plutella xylostella*) on Cabbage, *Indian J. Ent.* **59(4)**: 411–414.

Peter, C. D. M. P., Sundara Rajan, R. and Govinda Rajulu, (1989) Evaluation of cartap for the control of diamond back month, *Plutella xylostella* (L.) on cabbage, *Pestology* **13(12)**: 22–24.

Pimpert, M. P. (1990) Some future research directions for IPM in chickpea. A view point in chickpea in nineties, In: *Proc. of the 2nd Intl. Workshop on Chickpea (4–8 Dec. 1989)*, ICRISAT Centre: Andhra Pradesh, India, 151–163.

Shengal, U. K. and Ujagir, R. (1990) Effect of synthetic pyrethroids, neem extracts and other insecticides for the control of pod damage by *H. armigera* on chickpea and yield relationship at Patancheru in Northern India, *Crop Prot.* **9**: 29–32.

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Observations on the biology of *Cheiracanthium melanostoma* (Thorell) (Aranaeae: Clubionidae) occurring on cotton

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ABSTRACT: Studies on the Biology of *Cheiracanthium melanostoma* (Thorell) showed that the spider laid on an average of 2.5 cocoons in one season. The average incubation period was 8.9 ± 0.85 days and the number of spiderlings emerging per egg mass varied from 53–125 with an average of 89 ± 9.26. Males and females passed through 8 and 9 instars respectively to reach maturity. Male and female spiderlings took an average of 168.78 ± 3.09 days and 277.62 ± 9.70 days to complete the development from egg to adult. The total average life span of adult in the case of male and female 248.7 and 381.7 days respectively. The male and female mated readily under laboratory conditions. Mortality during different instars varied from 3.94 to 18.29 per cent. The female prepared the egg sac in a small concealed place by bringing together two broad leaves as a chamber with single entrance. It was also found to take care of the eggs by sitting over them.

KEYWORDS: Spiders, biology, *Cheiracanthium melanostoma*, cotton

It is commonly known that the spiders are normally insect eaters and that they spread their webs and capture their prey–insects. Web building spiders are usually ready to eat everything trapped in their webs. Hunting spiders attack any thing small enough that comes in their ways, without pausing to determine whether or not it has six legs and a head separated from its thorax. Instead of calling them an insectivorous, it is therefore a little more accurate to say that they are carnivorous and eat only living food.

Riley (1885) reported several species of spiders occurring on cotton and predaceous on the cotton pests. Fletcher and Thomas (1943) also reported spiders as predators of *Heliothis* larvae in cotton. Patel *et al.* (1986) studied spiders in cotton fields in Gujarat and reported 13 families of spiders on cotton.

Pollard and Jackson (1984) have studied the biology of *Clubiona cambridgei*. Likewise, Peck and Whitcomb (1970) have studied the biology of *Cheiracanthium*

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inclusum. *C. melanostoma* (Throell) was one of the dominant spiders found on cotton during the present study. In order to understand the biology of this predominant species, a study was carried out and the results are presented in this paper.

The present study was carried out during 1983–88. The females of *C. melanostoma* were collected from the cotton fields and kept in one liter glass jars. The mouth of the jar was covered with a fine nylon cloth held in a place by a rubber band. The females were fed laboratory reared larvae of *Corcyra cephalonica* Staint and *Spodoptera litura*. The eggs laid by such females were used for studying its incubation period and other biological characters. The studies were carried out at varying temperature of 25–30 °C and 60–80% relative humidity.

To study the incubation period of the eggs, the egg mass with the females were kept in one liter capacity glass jars. The mouth of the jar was covered with a piece of fine nylon cloth. The egg masses were examined daily and duration of hatching as well as number of spiderlings was recorded.

The newly hatched spiderlings were transferred into a clean glass vial (10 × 2.5 cm) using a fine camel hair brush and the mouth of the glass vial was plugged with a cotton plug. A cotton swab soaked in water was placed inside glass vial to maintain humidity. The spiderlings were fed with nymphs of aphids collected from fields for first few days. They were given 3–7 newly hatched larvae of *C. cephalonica* regularly until they reached the second instar. Thereafter 4–5 days old larvae of *C. cephalonica* (5–6 individuals) were given. Slightly grown up larvae were given as the development of spiderlings advanced. The food remains and excreta were removed every day and cotton swab was replaced every alternate day. The glass vials were also cleaned periodically in order to keep them clean. The development of spiderlings was closely observed. Measurements of male and female spiders were recorded using ordinary scale. Extent of mortality of the spiderlings at each instar was also recorded.

To study the mating behaviors, a pair of adult male and female was retained in one liter glass jar. A small twig of cotton plant was placed in jar in order to provide natural substrate. The adults were fed with larvae of *C. cephalonica*. A cotton swab soaked in water was also kept in the jar for maintaining humidity. Mating behavior and cocoon spinning were closely observed. Since parental care is often seen in spiders, close observations, were made to study this behavior of *C. melanostoma* in the laboratory.

The spiderlings emerged out from the tubular nest after the first moult, which took place inside the egg sac. The incubation period varied from 7 to 10 days, the average being 8.9 ± 0.85 days. The number of spiderlings emerged varied from 53–125 with an average of 89 ± 9.26 spiderlings per egg mass. Males completed their life cycle after 8 instars. However, females took an additional instar to complete their life cycle. Females completed their life cycle after 9 instars (Table 1). The spiderlings were fed with larvae of *C. cephalonica*. The male spiderlings of *C. melanostoma* took 163–300 days with an average of 168.78 ± 3.09 days whereas the female took 220–229 days with an average of 277.62 ± 9.70 days to complete the development. Total life span from the emergence of the spiderlings to the death of adult varied from 224–294 days in the case of males (average being 248.7 days) whereas it varied from 334 to 462 in

TABLE 1. Duration (in days) of instars and changes in carapace length, width and total length (in mm) of spider *Cheiracanthium melanostoma* (Thorell) reared in laboratory (average of 14 males and 15 females)

Instars	Number of days		Length of carapace		Width of carapace		Total length	
	Range	Mean	Range	Average	Range	Average	Range	Average
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Male ♂ (Female ♀)								
II	25–47 (24–46)	26.25 (25.17)	0.85–0.81 (0.65–0.92)	0.68 (0.70)	0.25–0.43 (0.31–0.51)	0.38 (0.42)	1.21–1.42 (1.41–1.59)	1.41 (1.51)
III	21–33 (21–36)	22.75 (31.67)	1.15–1.77 (1.26–1.81)	1.27 (1.36)	0.37–0.59 (0.41–0.69)	0.49 (0.59)	2.51–3.41 (2.95–3.57)	3.22 (3.48)
IV	11–33 (10–31)	21.27 (24.24)	1.65–1.85 (1.71–1.99)	1.77 (1.92)	0.55–0.99 (0.64–1.05)	0.89 (0.96)	3.21–3.99 (3.95–4.08)	3.81 (4.01)
V	16–38 (27–59)	23.67 (37.83)	1.81–2.01 (2.05–2.30)	1.99 (2.25)	0.98–1.35 (1.15–1.46)	1.15 (1.31)	3.81–4.51 (4.25–4.75)	4.46 (4.65)
VI	13–26 (16–41)	19.63 (29.65)	1.99–2.66 (2.21–2.56)	2.21 (2.42)	1.31–1.89 (1.38–1.99)	1.56 (1.75)	4.46–5.11 (4.71–5.20)	8.05 (5.15)
VII	26–51 (40–67)	29.55 (44.85)	2.55–2.89 (2.41–2.81)	2.37 (2.69)	1.67–2.15 (1.92–2.34)	2.11 (2.20)	5.05–5.75 (5.10–5.85)	5.66 (5.62)
VIII	16–41 (42–76)	25.66 (54.25)	2.81–3.07 (2.61–3.14)	2.99 (3.05)	2.10–2.52 (2.17–2.51)	2.43 (2.36)	5.59–6.81 (5.81–6.20)	6.80 (6.17)
IX	00–00 (15–41)	00.00 (29.96)	0.00–0.00 (3.00–3.37)	0.00 (3.21)	0.00–0.00 (2.47–2.76)	0.00 (2.60)	0.00–0.00 (6.15–7.26)	0.00 (7.03)

Total developmental period of ♂: 163–200 168.78, Total life span of ♂: 224–294 days, S.D: 3.09. Total developmental period of ♀: 226–299 277.62, Total life span of ♀: 334–462 days, S.D: 9.70.

the case of females (average being 381.7 days). The average length of adult male was 6.80 mm while it was 7.03 mm in the case of female.

Pollard and Jackson (1984) have reported similar developmental period for *Clubiona cambridgei*. Likewise Peck and Whitcomb (1970) have studied the biology of *Cheiracanthium inclusum* and reported identical findings. It was observed out of 57 spiderlings reared in the laboratory only 29 developed into adults. The per cent mortality during different instar varied from 3.94 to 18.29. The highest mortality (18,29%) was recorded in the last instar. On examining the dead spiderlings, it was found that spiderlings could not cast their exuviae and died. Peck and Whitcomb (1970) reported that moulting period is very critical in the life of spiders and great numbers do not successfully complete moulting. They also reported that if the spider failed to withdraw its legs from the old exuviae, it struggles for hours and finally dies. This might be due to a premature loss of moulting fluid.

Courtship was not a complicated process in *C. melanostoma*. The male came nearer to the tunnel shaped retreat of the female and tapped it to find whether the female was there or not. On finding the female, the male continued the tapping for some times with anterior leg and getting no response it approached her. The courtship lasted for 15–25 minutes. After a short struggle, she was quite and the male mounted on her back facing in opposite direction. It then moved over the female, tilted the female's abdomen and inserted its right palp into the right genital orifice and left palp into the left genital orfice alternately. The palps were introduced only once in each orfice. The whole activity of mating lasted for 15–25 minutes. After mating, the male moved away in search of other female, but before that it recharged its palps again. Males were found polygamous and females mated only once.

The abdomen of the female started swelling after successful mating and she started constructing the cocoon within 5–7 days. The female prepared the egg sac in a small, concealed place by bringing together two broad leaves or in curled leaves as a chamber with a single entrance. She deposited the eggs as a compact mass and covered with a thin sheet of silk. Cocoon was constructed only during night time. The silk thread was found coming out from the spinnerets and the spinning was done by the movements of the abdomen with the help of legs and palps. The mother guarded the cocoon till the eclosion. The female prepared 2–3 cocoons in one season (the average being 2.5 cocoons) with an interval of 10–30 days in between. The number of eggs in each cocoon varied from 53–125 (with an average of 96.5 ± 9.26 spiderlings). The female was observed to take care of the eggs. She was always found sitting close to the egg sac. However when a pray was offered, she left the egg sac and caught the prey. But immediately after catching the prey, she returned to the egg sac, and fed on the prey. Similar behavior was also observed in the field. This parental care was observed until the eggs hatched out. Spiderlings were left alone, after they hatched out from the egg sac.

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REFERENCES

Fletcher, R. K. and Thomas, F. C. (1943) Natural control of eggs and first instar larvae of *Heliothis armigera*, *J. Econ. Entomol* **36**: 560.

Patel, B. H., Pillai, G. K. and Sebastian, P. A. (1986) The ground activity of spiders in cotton fields in Gujarat, India, *Actas X Congr. Int. Arcon, Jaca/Espana* **1**: 245–251.

Peck, W. B. and Whitcomb, W. H. (1970) Studies on the biology of spiders *Cheiracanthium inclusum* HENTZ, *Univ. Arkansas. Agr. Exp. Sta. Bull* **773**: 76.

Pollard, S. D. and Jackson, R. R. (1984) Interpopulation variation in the life history of *Clubiona cambridgei* (Araneae: Clubionidae), a New Zealand hunting spider, *New Zeal. J. Zoo* **11(4)**: 413–416.

Riley, C. V. (1885) Cotton worm and boll, *Fourth. Rept. Us. Entomol. Comm.*, 399.

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